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Recruitment Limitation as a Consequence of Natural Selection for Use of Restricted Feeding Habitats and Predation Risk Taking by Juvenile Fishes

Carl J. Walters and Francis Juanes[1]

Fisheries Centre, University of British Columbia, Vancouver, BC V6T 1Z4, Canada

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Juvenile fishes generally have spatial refuges from predation, and forage in limited but risky areas near refuges. Models of food density dynamics within such limited foraging areas predict that food availability and consumption per time spent feeding should depend strongly on juvenile density. Selection should act on the time that juveniles spend foraging, so as to strike a balance between growth and predation risk; we predict that optimum balance will occur at foraging times proportional to the minimum needed to reach viable sizes for later survival and reproduction plus an additional time inversely proportional to the predation risk per time. Combining the food availability and optimal foraging time predictions leads to stock–recruitment patterns similar to classic Beverton–Holt and Ricker forms, depending on how food organisms respond over time. Very strong density-dependent mortality can occur even without noticeable changes in juvenile growth rates or average food densities over large spatial scales, but intraspecific competitive effects should always be evident through density-dependent changes in foraging time.

Les poissons juvéniles disposent généralement de refuges qui les abritent de la prédation, et s'alimentent dans un périmètre limité mais risqué près des refuges. Des modèles de la dynamique de la densité de nourriture dans ces zones limitées prévoient que la disponibilité de nourriture et la consommation en fonction de la durée passée à s'alimenter doivent dépendre fortement de la densité des juvéniles. La sélection doit agir sur le temps passé par les juvéniles à se nourrir, de façon à établir un équilibre entre la croissance et le risque de prédation; nous prédisons que l'équilibre optimal se produira pour des durées de périodes d'alimentation proportionnelles au minimum nécessaire pour atteindre des tailles viables permettant la survie puis la reproduction, plus une période additionnelle inversement proportionnelle au risque de prédation en fonction du temps. En combinant les prédictions sur la disponibilité de nourriture et la durée optimale de la période d'alimentation, on obtient des schémas de recrutement des stocks similaires aux formes classiques des modèles de Beverton–Holt et Ricker, qui dépendent de la façon dont les organismes proies réagissent en fonction du temps. On peut observer une mortalité très forte dépendante de la densité sans même qu'il se produise de changement notable dans les taux de croissance des juvéniles ou les densités moyennes de nourriture sur de grandes échelles spatiales, mais les effets de la compétition intraspécifique doivent toujours se manifester par des changements de la durée de la période d'alimentation qui dépendent de la densité.

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Food competition and predation have long been suspected to limit recruitment rates in fishes, but field evidence to support or refute these suspicions has proven very difficult to find (Leggett 1986; Houde 1987, 1989b). Often, total recruitment appears to vary strongly and to be, on average, independent of adult spawning stock or egg deposition. However, even in such cases there must be strong density-dependent changes in average larval or juvenile survival rate to prevent recruitment from showing some increasing trend with increasing egg deposition. Average recruitment levels resulting from such density-dependent changes are seldom correlated in any obvious way with food production or predator abundance (see reviews in Rothschild 1986; Anderson 1988; Daan et al. 1990; Heath 1992).

We develop a series of models to show that the lack of simple correlation between food, predators, and recruitment could result from natural selection for restriction of foraging time in habitats with high predation risk. We show first that food densities and consumption per time foraging in restricted foraging habitats can depend strongly on juvenile densities even when juveniles are not sufficiently abundant to affect large-scale food densities or production. We then show that natural selection should favor increasing foraging time and hence predation mortality as juvenile density increases, so that mortality rates can be density dependent even without food competition effects being exhibited through changes in growth rate. We examine the form of stock–recruitment relationships resulting from such interactions and show that forms like the Beverton–Holt and Ricker can result from differences in the way juveniles respond to changes in food density. We conclude that investigation of how density affects the way juvenile fishes distribute themselves on very small space and time scales is critical to understanding stock–recruitment relationships and trophic interactions.

It is certainly possible for recruitment limitation to arise from lack of suitable spawning habitat or mechanisms that cause density-dependent and/or density-independent mortality of eggs

[1]Present address: Department of Forestry and Wildlife Management, University of Massachusetts, Amherst, MA 01003, USA.

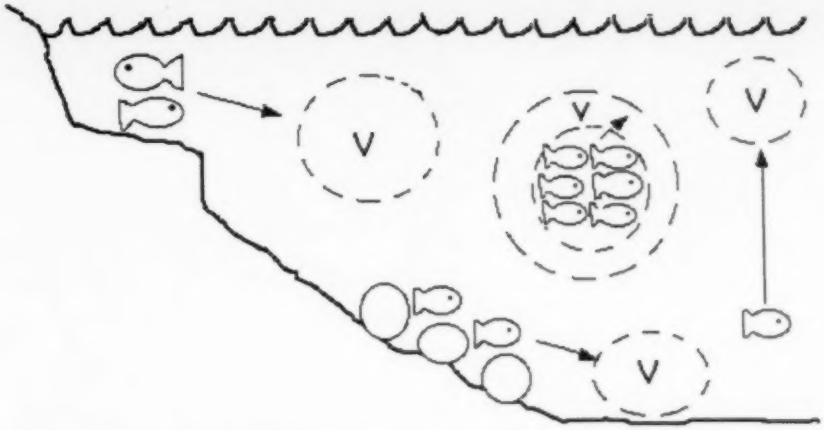

FIG. 1. Juvenile fishes use a remarkable variety of spatial refuges from predation and may be restricted to limited foraging volumes *V* near these refuges. Author's son William Walters, age 9, was able to identify several and produce this illustration, even with his limited fish experience.

and larvae. We restrict attention here to the later period in juvenile life during which individuals (1) have at least some limited control of their movements, allowing habitat choice, and (2) are small enough to face potentially high predation risk and are not yet capable of reproduction. Various workers have suggested that an understanding of the ecology and dynamics of juvenile stages should be an integral part of assessing and managing fish populations (Rothschild 1986; Houde 1987; de Lafontaine et al. 1992), particularly because it appears that the early juvenile phase can be critical in determining future year-class strength (Nielsen 1980; Sissenwine 1984; Peterman et al. 1988; Hallowed and Bailey 1989; Wooster and Bailey 1989; Szedlmayer et al. 1990; Bailey and Spring 1992;Bradford 1992). The important point is that even if density-dependent losses have acted in a major way on earlier stages, the mechanisms discussed below could still act to further reduce recruitment or to "override" the earlier limits, and hence to substantially dampen variability caused by environmental factors (as suggested by Gulland's (1965) idea of coarse and fine control of year-class strength variation). This effect has been shown for North Sea plaice stocks (see Beverton and Iles 1992), where although year-class strength is roughly determined ("fast coarse" control) during the pelagic larval phase (Rauck and Ziljstra 1978; Ziljstra and Witte 1985), a further readjustment ("fine" control) occurs early in the postsettlement phase (van der Veer 1986; van der Veer et al. 1990; Pihl 1990) when juveniles experience strong density-dependent mortality (Lockwood 1980; van der Veer 1986; ve der Veer and Bergman 1987; Pihl 1990; Rijnsdorp and van Leeuwen 1992).

Prediction of Food Availability in Restricted Foraging Areas

Juvenile fishes generally spend most of the daylight hours in restricted spatial refuges where they are not highly vulnerable to visual predators (Helfman 1993). These refuges usually offer few or no feeding opportunities, and usually juveniles only move out from them to feed during short periods near dawn and dusk (e.g., diel vertical migration in juvenile sockeye salmon (*Oncorhynchus nerka*), Clark and Levy 1988) or at night (e.g., nocturnal inshore movement and feeding by juvenile cod, Atlantic cod (*Gadus morhua*), Robb 1981; Pihl 1982; Clark and Green 1990; Keats 1990). The remarkable diversity of refuges used by juvenile fish (Fig. 1) hints at how strong the selection pressures must be to favor such behavior: some gather in very shallow water (Gibson 1993), others in the darkness of deep water (Neilson and Perry 1990; Greenberg and Stiles 1993; L'Abée-Lund et al. 1993), and still others find hiding places in rocky substrates or reef structures (Keats et al. 1987; Lough et al; 1989; Ebeling and Hixon 1991; Helfman 1993), and many species gather into tight shoals or schools where each individual spends most of its time with many neighbours between itself and the hungry mouths (Pitcher and Parrish 1993). For example, grunts spend the day schooling inactively on coral reefs, and after dusk migrate to nearby seagrass beds to feed (Ogden and Ehrlich 1977). The timing of these feeding migrations is strongly influenced by the activity of lizardfish, the primary piscivore in this system (Helfman 1986). The rapidity with which small fish vacate dangerous habitats when predators are experimentally added to a system (Werner et al. 1983; Schlosser 1987; He and Kitchell 1990; He and Wright 1992) and the increased survival of prey fish that is observed when refuge availability increases or predator density decreases (reviewed in Hixon 1991) suggest that selection must favor not only strong avoidance behavior in general, but also flexibility to vary its use.

But the use of such refuges implies very tight limits on the volume (or area) of potential feeding habitat available to juvenile fishes during those periods when they do move out (or as schools break up to feed in the twilight hours). Moving further or spending more time moving both imply increasing predation risk. To simplify the following discussion, we will treat the accessible feeding habitat volume (V) as fixed and consider only the effects of variation in the time spent in this volume; similar predictions are obtained for some more complex models where accessible habitat volume is admitted to vary with time spent feeding and juvenile size (swimming speed), but the impacts of varying juvenile density on food density (and hence growth per time feeding) are more difficult to calculate and visualize for such models. Below, we will sometimes refer to the refuge habitat and nearby foraging volume V together, calling this combination the juvenile "nursery habitat."

We now develop two extreme models for the effect of juvenile

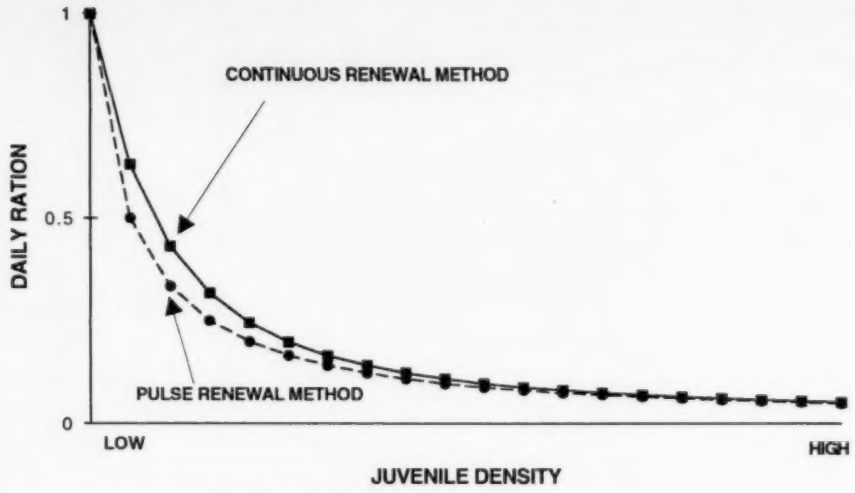

FIG. 2. Predicted effect of juvenile density on daily ration within restricted foraging volumes near predation refuges. Pulse and continuous renewal models represent alternative temporal patterns of delivery of food organisms to the foraging area.

density and time spent in the feeding volume V on the biomass density B of food organisms and resultant daily rations. For both, we assume that (1) food densities are generally low enough that handling time and satiation effects on daily rations can be ignored and (2) prey are widely enough distributed within V that juveniles cannot simply sit in one place and feed to satiation (the prey may be randomly distributed or clumped within V but we assume that juveniles are unable to hold in place and feed very efficiently for a long time on any clump they find). Under these two assumptions, daily rations F per fish should be proportional to the effective volume v swept per juvenile per time spent feeding (dependent on swimming speed, reaction distance to prey, and capture success), proportion of time spent feeding R, and prey biomass density B. If prey density does not vary over the feeding period, the per capita daily ration would then be just $F = vRB$.

Consider first a "pulse renewal" model, where food biomass is assumed to be renewed to a level B_0 each day during the time when juveniles are not feeding, through processes such as dispersal or migration of food organisms into the feeding volume V, water mixing processes, and food life history events like pupation. Processes such as these may well make B_0 totally independent of the number N of juvenile fish, at least if V is small compared with the volume (or area) over which longer term food production processes (growth, reproduction) occur. To simplify the algebra, let us assume further that R is small enough each day that we can ignore prey loss processes other than juvenile fish feeding during the feeding period(s). Then the biomass dynamics of B during the feeding period will be given by

$$(1) \quad dB/dt = -(v/V)NB$$

where v/V represents the instantaneous rate of food organism mortality per juvenile fish N. Note that the total mortality rate $(v/V)N$ can be large due either to (1) high v per fish (unlikely for small fish), (2) small V, or (3) high juvenile density N. The solution to eq. (1) is the familiar exponential decay equation, and the predicted total food consumption over the period R by juveniles is just $VB_0(1 - \exp[-(v/V)NR])$, i.e., the volume times the change in food biomass density per volume. The daily ration per juvenile will be this total consumption divided by N:

$$(2) \quad F = VB_0(1 - \exp[-(v/V)NR])/N.$$

This model predicts exponential or hyperbolic decrease in daily ration F with increasing N (or N/V) as shown in Fig. 2, with F being most sensitive to N when N is low. The basic mechanism in this case is exploitation competition during the feeding period R.

As an extreme alternative, consider the "continuous renewal" model where food enters the volume V continuously at a rate E and is lost at an instantaneous rate m in addition to the loss rate due to fish feeding:

$$(3) \quad dB/dt = E - mB - (v/V)NB.$$

E may involve biological processes such as insect pupation and emergence as well as physical mixing processes carrying food organisms into V from some larger production environment. If E and m are fairly large (as could occur for example due to physical mixing processes in a small volume where juveniles feed on plankton), then this model predicts that B will quickly reach equilibrium at a concentration B_e, where

$$(4) \quad B_e = E/[m + (v/V)\,N]$$

and daily ration per fish is predicted to be $F = vRB_e$. Again, this model predicts a hyperbolic pattern of decrease in daily ration with increasing N/V (Fig. 2), with the largest effects occurring at the lowest juvenile densities.

Above, we treat R as fixed and predict food density B and consumption F as functions of fish density N; thus, we have ignored satiation effects. An alternative approach would be to assume that juveniles can generally feed to satiation (until they

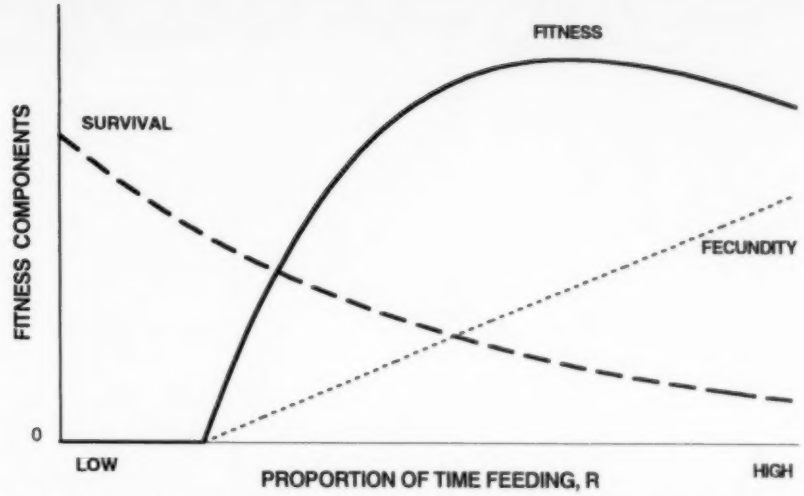

FIG. 3. Fitness as a function of proportion of time spent feeding. Survival decreases with increasing time due to predation risk, while long-term survival–fecundity increases only when some minimum or threshold feeding time is exceeded. Note that threshhold time may be strongly density dependent.

achieve some target F) and to predict the foraging time R needed to achieve this satiation. In this approach, we would still conclude that N affects B and hence the time R needed to achieve satiation. The basic effect is the same in either case: as N increases relative to the available foraging volume V, juveniles must either accept lower F or increase R to maintain the same F.

The key point of these models is that it is flatly misleading to assert that competition for food should have little effect when juvenile abundance is low. Daily rations should in fact be most sensitive to abundance *changes* when abundances are low and will depend entirely on (1) how large the volume V is where the juveniles compete and (2) renewal rates of food into this volume. Moreover, the models above predict that competitive effects can be large even when juvenile feeding has no long-term effect on prey abundance through prey population dynamics mechanisms (i.e., overexploitation of some prey species) and replacement of preferred prey types with species/morphs that are less vulnerable to predation. It is of course possible for vN to be so small relative to V that competition effects are insignificant.

The above models link daily ration F to juvenile density N and proportion of time spent feeding R. In the next section, we discuss the relationship between R and lifetime fitness, which presumably depends on the size that juveniles reach before leaving refuge areas and/or how long they take to reach some minimum viable size for emergence. Before proceeding to the fitness discussion, we thus need to point out the functional linkage between F, R, and size predicted by basic bioenergetics models (Beyer 1989; Adams and Breck 1990). One simple representation of this linkage is the density-dependent growth model of Walters and Post (1993). That model combines eq. (4) with the assumptions that (1) search voume v varies as roughly the square of body length L and (2) metabolism of small fish is approximately proportional to body weight (Giguere et al. 1988; Houde 1989a). These assumptions result in the growth model

$$(5)\quad dL/dt = k_1 R / (1 + k_2 L^2 N) - k_3 L$$

where the constants k_1 and k_2 combine the food turnover parameters of eq. (4) with the length–weight parameters and bioenergetics parameters for assimilation efficiency and specific dynamic action, while k_3 represents effects of routine metabolism (for details of the derivation, see Walters and Post 1993). Numerical integration of equations like (5) over time, under various assumptions about how N changes during the juvenile stage, leads to the prediction that the foraging time R required to reach a given threshhold size L_0 should be roughly proportional to the initial density N_0 when the juveniles begin to use the refuge habitat. This rough prediction breaks down if L_0 is close to the maximum size that the juveniles can reach even when N_0 is low; in such cases, the R required to reach L_0 within some reasonable time horizon may increase exponentially with N_0. However, such exponential increase would simply exaggerate the effects predicted below from simpler proportional increase.

Prediction of Optimal Foraging Time

Here we show that natural selection for behaviors that optimize the risk taking time R should very generally result in density-dependent juvenile mortality. The key conditions for this prediction to hold are shown to be (1) existence of a minimum or threshhold foraging time R_0 below which the juvenile can never successfully leave the nursery habitat and (2) heritable variation in behavioral traits that result in variation in R with changes in food density. There is a substantial literature on how predation risk should influence optimal foraging bahavior and growth (i.e., reviews by Helfman 1986; Sih 1987; Gilliam and Fraser 1988; Godin 1990; Lima and Dill 1990: Werner 1992: Milinski 1993); our presentation here is really just a review and restatement of those findings, with linkage to the predictions of the previous section.

The expected lifetime fitness W of a juvenile fish beginning at the time it enters the juvenile nursery habitat can be expressed as

$$(6)\quad W = S_j S_m \bar{f} = S_j f$$

where S_j is survival rate through the juvenile stage, S_m is survival to maturity after leaving the juvenile nursery, and f is adult fecundity (or sum over mature ages of survivorships from maturity to age times fecundities at age for iteroparous species). f merely combines survival and fecundity effects after leaving the nursery: $f = S_m f$. Both S_j and f presumably vary with R. Let us assume that predation loss simply involves random encounters with predators during feeding times (no complex predator behavioral responses such as type III switching or satiation at high juvenile densities); then we expect S_j to vary as

$$(7) \quad S_j = \exp\{-M_0 - M_1 R\}$$

where M_0 is a base natural mortality rate that would occur even in the absence of predators, and M_1 is the instantaneous predation risk per time spent foraging. In this case, it is easily shown by differentiating eq. (6) with repect to R that W is maximized at that R value R_{opt} where

$$(8) \quad (df/dR)/f = M_1,$$

i.e., fitness is maximized at the feeding time "investment" where the relative gain in long-term survival–fecundity $(df/dR)/f$ is just balanced by the predation risk per time feeding.

Suppose next that long-term survival–fecundity f is an increasing function of feeding time R (increased feeding time generally leads to larger body sizes (Wootton 1990), and fecundity and survival are positively related to body size in fishes (Bagenal 1978; Peterson and Wroblewski 1984), for R above some minimum threshhold R_0 necessary to successfully emerge from the refuge, survive, and/or reproduce at all (see Fig. 3). The exact form of f is not critical to our arguments, so for convenience, let us assume that f is linear:

$$(9) \quad f = k_f \cdot (R - R_0) \text{ for } R > R_0 \text{ and } f = 0 \text{ for } R < R_0$$

where k_f is the slope of the f versus R relationship for $R > R_0$. For this functional form, application of eq. 8 leads to a very simple prediction for the optimum feeding time:

$$(10) \quad R_{opt} = R_0 + 1/M_1,$$

i.e., fitness is maximized by spending a proportion of time spent feeding equal to the bare minimum needed to survive/reproduce after leaving the juvenile nursery, plus additional time inversely proportional to the predation risk M_1 per time spent feeding. Remarkably, the optimum time is completely independent of the absolute slope k_f of the long-term survival–fecundity relationship, i.e., it does not matter how rapidly survival–fecundity improves for feeding times greater than R_0. We have checked the eq. (10) prediction for a variety of more complex f functions (polynomial, logistic, etc.), and it appears to be very robust. An interesting special case is $R_0 = 0$ (any nonzero feeding time leading to proportional increase in long-term fitness); for this case, $R_{opt} = 1/M_1$, i.e., the optimum feeding time would depend only on predation risk and would be entirely independent of density (since R_0 is related to juvenile density as shown in the previous section) except insofar as M_1 might vary with density through effects such as attracting predators to the feeding volume or long-term increase in the number of cannibalistic adults.

Note that eq. (10) can predict $R_{opt} > 1$ for low values of predation risk M_1 and/or high values of R_0. In this case the optimization is predicting that juveniles should either feed all the time or extend the total time spent in the nursery habitat, as in models for the timing of ontogenetic habitat shifts (Werner and Gilliam 1984; Werner and Hall 1988). We do see such extensions, at least when R_0 is large; for example, anadromous salmonids rearing in very unproductive lakes/streams tend to spend more years in freshwater, and the proportion smolting at younger ages sometimes appears to be lower when densities are high (see reviews in Groot and Margolis 1991). Growth rates, rather than just threshold smolting size, can also determine smolting age so that faster growing parr smolt younger and smaller than slow-growing parr (Okland et al. 1993). However, if only M_1 is very low, it is questionable whether a refuge-use strategy would be optimal in the first place; we suspect that very few (if any) juvenile fish enjoy this circumstance under natural conditions. However, refuge-use tends to be minimized when predation risk is low. For example, shoaling tendency decreases with increasing size in goatfish (Uiblein 1991), in many species, shoals become more compact and cohesive when predators are detected or in daylight (Luo 1993; Pitcher and Parrish 1993), shelter affects goby distributions only in the presence of predators (Utne et al. 1993), and diel migrations of various juvenile fish are less intense in turbid lakes than in clear lakes (likely related to the visual searching ability of the dominant piscivores) (Levy 1990; Gliwicz and Jachner 1992).

We can now state the key result of our analysis: if the minimum feeding time R_0 needed for successful emergence from the juvenile nursery is density dependent (and it should be strongly so based on the arguments of the previous section), then selection for optimization of feeding time according to eq. (10) should lead to density-dependent juvenile mortality. That is, juvenile survival $S_j = \exp\{-M_0 - M_1 R_{opt}\}$ should be a decreasing function of juvenile density because R_0 should increase with increasing density. Young-of-the-year steelhead (*Oncorhynchus mykiss*) show density-dependent dispersal, growth, and survival. Overwinter survival is contingent upon fish reaching a threshold weight, and the proportion of fish attaining the threshold size is inversely related to age-0 density (Close and Anderson 1992). Although the authors did not examine the mechanisms causing density-dependent threshold sizes, the arguments presented above would suggest that the main cause may be density-dependent changes in R_0.

The density-dependent survival effect may or may not be accompanied by noticeable changes in growth rate or juvenile size at the time of emergence from the nursery. If the predation risk M_1 during juvenile feeding is high, then the optimum feeding time will be near R_0 and juveniles should always emerge at or near the minimum viable size after electing not to take the additional risks needed to improve growth rate. Note that this conclusion is not affected by arguments about how increased growth rates and size may lead in the long term to reduced predation risk; if the feeding time required to achieve increased size is density dependent, then the overall effect on juvenile survival will still occur even though increased size contributes to higher f values later in life.

In the derivation of eq. (7)–(10) we have treated predators as a proximate or incidental source of mortality, with an overall impact on juvenile survival dependent on how feeding conditions affect time exposed to predation risk. This assumption is certainly parsimonious, and is probably the most realistic for most juvenile fishes, who face a wide suite of potential predators and are rarely numerous enough to cause strong predator

handling time or satiation effects (predator stomach contents data rarely suggest satiation for long periods of time on any one type of juvenile fish; see reviews in Dill 1983; Wootton 1990; Yamaoka 1991). But there are two complications worth mentioning. First, some predators may "switch on" or aggregate in juvenile feeding areas when juvenile densities are high (Mittelbach and Chesson 1987); this would simply exaggerate the density-dependent effects predicted earlier. Second, in some situations, especially with schooling juveniles, short daily feeding periods may combine with efficient predator searching and relatively long handling times per prey to produce predation rates that are depensatory at high juvenile densities. That is, predators may each take a nearly constant number of juveniles per feeding period, so that the instantaneous predation risk M_1 varies as

$$(11) \quad M_1 = mRP/N$$

where m is the daily number of juveniles eaten per predator, P is predator abundance, and N is juvenile abundance. This inverse dependence of risk on N could result in selection for longer feeding times R and ultimately lead to essentially density-independent net predation effects (R proportional to N due to food competition would make M_1 in eq. (11) independent of N). However, it is hard to imagine such happy situations for the predators lasting very long, due both to eventual decreases in N and to responses of predator abundance given an essentially unlimited food supply.

Another possible objection to the survival model (eq. (7)) leading to the fitness-maximizing prediction of eq. (10) is that incidental predation risk may increase more violently with R than is assumed in the proportional model M_1R. This would be expected for example in situations where juveniles feed mainly near dawn and dusk, so that feeding a bit longer in the morning or starting earlier in the evening means moving around when light levels are higher and visual predators can search larger water volumes (effects of light level on reaction distances, see Confer et al. 1978; Zaret 1980; Henderson and Northcote 1985). If such effects lead to harsh selection against increasing R, then at high densities, juveniles may have little choice but to grow more slowly and take longer to reach sizes where they can move out of the nursery habitat. The basic selection model for this case is the same as presented above, but with R replaced by a variable representing total time spent in the nursery. This model predicts strong changes in both growth and survival with density, as in the recruitment models proposed by Ware (1975, 1980), Shepherd and Cushing (1980, 1990), and Beyer (1989) and in the individual-based models of DeAngelis et al. (1991, 1993). Thus, previous recruitment models based on density-dependent growth and time exposed to size-selective predation may be viewed as a special or limiting case of the theory presented here, for situations where M_1 is very large.

Foraging Time Decisions and Stock–Recruitment Relationships

While the analyses presented above indicate that we should very generally expect to see some pattern of density-dependent mortality in juvenile fish, they unfortunately do not lead directly to simple predictions about the functional form of the stock–recruitment (or more precisely, the larval settlement–recruitment) relationship. Such predictions require examination of at least two additional relationships involving space–time scales that we have so far ignored: (1) how juveniles adjust their risk-taking behavior over the rearing period, as densities decrease and body size increases and (2) how density-related changes in larval dispersion pattern, and juvenile dispersal behaviors, are related to the mesoscale spatial structure of nursery habitats (where we assume that the feeding volume V most often consists of a set of localized subvolumes V_i that vary in food availability and predation risk). In the following discussion, we will refer to "recruitment" as the number N_r of juveniles that successfully emerge from the juvenile nursery; if N_0 fry or larvae enter the nursery, then $N_r = N_0S_j$. Obviously, we could extend the prediction using S_m of eq. (6) to predict recruitment of mature fish, although we would need to be careful about whether density-dependent variations in optimal body size at emergence might lead to further density dependence in S_m (e.g., density-dependent decreases in smolt size of sockeye salmon are probably associated with decreases in subsequent marine survival; see review in Burgner 1991).

There are at least three alternative possibilities for how juveniles might adjust their foraging times in relation to density-related changes in food availability: (1) no individual adjustment — changes in foraging times result from density-dependent selection for animals with different foraging time phenotypes; (2) early adjustment — foraging time for each individual is determined by the competitive conditions that it encounters early in life; (3) continuous adjustment — juveniles make rapid adjustments in foraging times as densities change during the development of each cohort. The following subsections discuss the widely differing predictions arising from each of these possibilities, and further subsections discuss the implications of mesoscale structure in nursery habitats and the use of stock– recruitment equations as empirical descriptions for the net effects of behavioral responses.

No Individual Adjustment of Foraging Time

Suppose each individual i in a population has a fixed foraging time phenotype R_i, and does not adjust foraging behavior to changing competitive conditions. Suppose further that there is heritable variation in R_i among individuals. These are the classical conditions for density-dependent selection. At high population densities, selection should favor "K-type" animals with high R values (R_0 high if density high); at low densities, selection should favor "r-type" animals with low R values (less foraging time needed, so avoid predators instead). In this case, it is meaningless to talk about a single stock–recruitment relationship because the relationship should change over time as selection alters the mix of low-R and high-R type individuals in the population, and hence the juvenile mortality rates due to predation. Such response delays have some very frightening implications for what we might see in the field under varying expoitation regimes.

Consider a population that has stabilized at a high level, such that competition among juveniles is intense and selection has favored relatively high R values. Now suppose that exploitation rate increases such that egg production and recruitment of juveniles to the nursery habitats decreased greatly. These juveniles will initially be mostly high-R types and will show substantial improvements in growth rate; meanwhile, juvenile survival rates will remain relatively low (due to high R and associated predation), and recruitment rates may decline. But selection will now favor individuals who spend less time foraging, so over time the average R value will decrease and growth

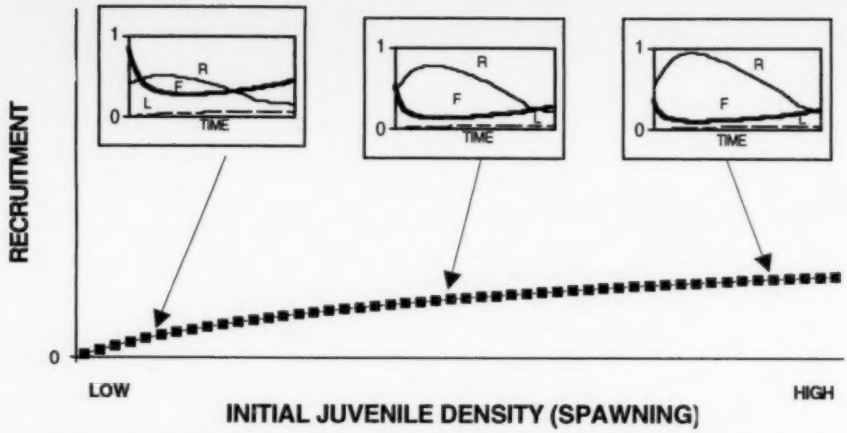

FIG. 4. Simulated stock–recruitment relationship for a population where juveniles vary foraging time during development by following a myopic decision rule. Inset graphs show sample time trajectories over the juvenile rearing period of body length (L), food density (F), and proportion of time spent feeding (R).

rates will fall back toward the pattern seen before the population decline; during this period, juvenile survival rates will improve, tending to stabilize total recruitment. Precisely this sort of density-dependent improvement but later decline in growth rates has been seen in major Atlantic cod stocks but has been attributed either to ecosystem changes or to selection against fast-growing fish by the fisheries (Hanson and Chouinard 1992); recruitment rates also appear to have declined and then stabilized as we predict. Although juvenile Atlantic cod are known to be sensitive to predation risk (Nordeide and Svasand 1990; Gotceitas and Brown 1993), the flexibility and heritability of predation risk behavior remain largely unexplored (but see Johnsson and Abrahams 1991 for an example of inherited predation risk behavior).

The density-dependent selection scenario becomes even more frightening when we ask what should happen when fishing is stopped to allow stock recovery, as has recently occurred with the Northern cod stock off Canada's east coast. If the stock has been kept down long enough for growth rates to decline, signalling predominance of low-R type juveniles, then increases in spawning associated with the fishery closure will at first cause increases in the density of competing juveniles, causing growth rate declines and possibly other signals of recruitment limitation (delays in leaving nursery habitats, reduced survival S_r to recruitment). Selection will now begin to favor high-R juveniles, but there may be considerable delay before these individuals become abundant enough to result in high total recruitment rates. In short, the linkage of the selection process with population increase may cause the increase to be drastically delayed compared with what we would predict from simple stock–recruitment relationships based on recruitment data obtained during the population decline.

One might object at this point that the whole argument about low-R and high-R individuals is silly because surely natural selection should favor at least some degree of behavioral plasticity (alteration of R by individuals, with changing competitive conditions) of the types discussed below. But is behavioral plasticity always favored, especially in long-lived species like the Atlantic cod that have probably been very abundant for a long time? Under such circumstances, it is entirely possible that selection has indeed favored specialized genotypes rather than flexible phenotypes (Real 1980; Van Tienderen 1991). For example, even diet generalists such as bluegill (*Lepomis macrochirus*) have evolved relatively inflexible habitat-specific foraging behaviors (Ehlinger and Wilson 1988; Ehlinger 1990).

Early Adjustment of Foraging Time

If juveniles are slow to adjust their risk-taking behaviors with changes in the density of conspecific competitors, then they may persistently act as though R_{opt} in eq. (10) is high if N_0 was high at the time when they began to use the nursery. If R_{opt} is roughly proportional to N_0, the overall recruitment relationship will then be a dome-shaped or Ricker form:

$$(12)\quad N_r = N_0 \exp\{-M_0 - M_1 R_{opt}\} = N_0 \exp\{-M_0 - M_1' N_0\}$$

where M_1' is M_1 times the proportionality constant between R_{opt} and N_0. The exponential effect could of course be exaggerated by increases in M_1, if increases in N_0 are associated with increases in number of cannibalistic older fish.

Continuous Adjustment in Foraging Times

At the opposite extreme, juveniles might adjust their daily times at risk rapidly as available food density varies with body size and N (eg., if it varies inversely with L^2N as in eq. (5)). If mortality rate then ends up being roughly proportional to N remaining at each time during the juvenile stage, the overall recruitment relationship will be precisely the asymptotic form known as the Beverton and Holt relationhip (Shepherd and Cushing 1980). However, the mechanism need not be so simplistic; an asymptotic relationship can arise from rapid behavioral adjustments even if prey availability and foraging time vary in a complex way over time due to changes in both juvenile lengths and abundance. Figure 4 shows an example of this situation. To construct the example, we simulated food density and growth changes over a hypothetical rearing period using eq. (3) and (5) while assuming instantaneous predation mortality rate each day proportional to R treated as a daily proportion of time spent feeding. R was varied over time by

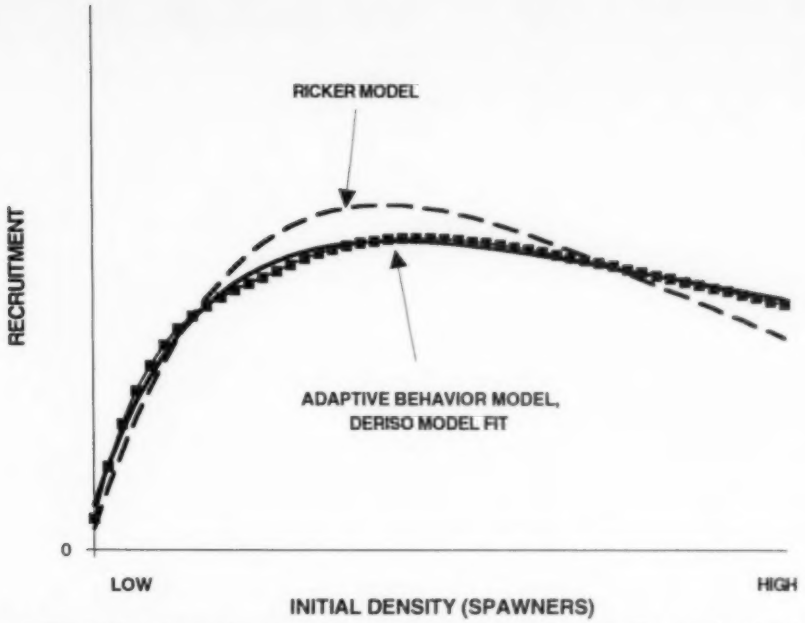

FIG. 5. Simulated stock–recruitment relationship for a population where juveniles vary foraging time during development in relation to both changing food density and predation risk, where predation risk is assumed due to cannibalism and is proportional to initial juvenile density. Fitted equations are the Ricker model and the recruitment model of Deriso (1980).

assuming that the fish follow a "myopic decision rule," choosing an R each day that would optimize fitness if food densities remained the same after that day (over the rest of the rearing period). Note in Figure 4 that such a behavioral decision rule would result in the juveniles spending too much time feeding early in the juvenile period (when L^2N is still high), but then stopping entirely later when they "discover" that food supply has recovered due to reduced N.

A fascinating question is whether natural selection could result in the evolution of decision rules that avoid the overcompensation in R shown in Figure 4, to somehow make the juveniles act as though they were able to predict future changes in competitive conditions during the rearing period. Such optimal decision rules can be computed using dynamic programming (Mangel and Clark 1988) and compared with observed trajectories in feeding times.

Suppose that cannibalism is the main cause of M_1 and that individuals can adjust rapidly to both changing food densities and predation risks. In this situation, we would expect N_0 and M_1 to be positively related (in the simplest case, proportional to adult density), and the myopic decision rule used to produce the results in Fig. 4 leads instead to a dome-shaped recruitment relationship similar to the Ricker curve (Fig. 5). However, we simulated this situation for a variety of growth–mortality parameter combinations, and all produced a characteristic pattern of deviations from the classic Ricker form: higher recruitment rates at very low stock sizes than predicted by the Ricker model, along with a flatter dome and less pronounced decrease at high stock sizes. These differences are perhaps trivial from a management point of view, and are certainly not helpful in terms of recruitment forecasting considering other sources of recruitment variation, but nevertheless represent a "signature" that might be useful in empirical studies aimed at determining whether adaptive changes in juvenile behavior may be influencing recruitment patterns.

Mesoscale Structure in Nursery Habitats

If the overall juvenile rearing environment consists of a series of sites with feeding volumes N_i varying in size or quality (maximum prey density), recruitment prediction is complicated by questions of whether juveniles can somehow find and occupy better areas first and whether selection will favor behavioral decision rules for dispersal (MacCall 1990). Assuming that dispersal between sites involves the same kind of predation risks as feeding, we can obviously construct selection models that imply density-dependent dispersal mortality (i.e., do not move unless your current site is crowded enough to prevent successful growth through adjustment of R, or balance risks associated with variation in R against the odds/risks of finding a better site). Depending on habitat structure and the riskiness of dispersal, such models might predict very complex recruitment relationships. One possibility is apparent multiple equilibria or "plateaus" in recruitment if dispersal is very risky; in this case increases in density up to some threshhold point may result in increased foraging time and hence a flattening of the recruitment function, but accidents that push density beyond the threshold may lead to dispersal that in turn results in a higher plateau due to colonization of more habitat sites. Another possibility is an "ideal free" situation where all individuals achieve the same R through dispersal (Fretwell and Lucas 1970; Fretwell 1972).

Empirical Equations for Stock–Recruitment

Various behavioral response lags could produce recruitment relationships intermediate between the Ricker and Beverton–Holt extremes. Such a spectrum of outcomes is predicted by the recruitment model of Deriso (1980), which Schnute (1985) showed is conveniently parameterized as

$$(13) \quad N_r = aN_0 (1 - bcN_0)^{1/c}$$

where a, b, and c are parameters to be determined empirically (Fig. 5). Deriso (1978) derived this function from a two-variable model for the joint time dynamics of juvenile numbers and biomass of prey, where the prey are assumed to have density-independent population growth rate and are eaten at a constant rate per juvenile fish, while instantaneous mortality rate of juvenile fish is assumed proportional to the ratio of fish to prey densities. Although his submodel for prey dynamics is quite different from the ones we suggest above, that submodel could be interpreted as representing how juveniles perceive or measure changes in prey density (i.e., with behavioral response lags). Further, his ratio-dependent mortality term can be interpreted as one simple way to predict the outcome of time-dependent risk-taking behaviors associated with both feeding and dispersal. Thus, we predict that Deriso's formula will turn out to be a good way to empirically represent the varied recruitment patterns that might result from selection on R (Fig. 5). Another expression that may do a good job at empirically representing delay effects is the one proposed by Gomez Muñoz (1986).

Discussion

Although there is nothing really new in the arguments presented above, our analysis has brought together disparate models to yield new insights into how individual behavioral mechanisms might lead to whole-population dynamics. Biologists have long speculated that intense food competition may arise from restriction of feeding in small areas, whatever may cause the restriction (e.g., see Mittelbach and Chesson 1987; Persson and Greenberg 1990; Persson 1991). Previous models analyzing the trade-off between predation risk amd energy gain have focused on the adaptive control of foraging effort as it relates to the forager's life history (Abrams 1991), various individual responses in activity level related to the presence of predators (reviewed in Werner 1992), trade-offs mediated through habitat choice (Werner and Gilliam 1984; Gilliam and Fraser 1987; Brown 1988; Abrahams and Dill 1989; Ludwig and Rowe 1990), the effects of uncertainty in predation risk on optimal patch use (Sih 1992), and the prediction of population bottlenecks when predation risk exceeds species-specific maximum potential reproductive rates (r_{max}) (Williamson 1993). Density dependence has usually been incorporated into such models using notions of the ideal free distribution (Rosenzweig 1991) or to predict the joint effects of changing resources and predators on habitat choice of competing species (Gilliam and Fraser 1988).

What is new in our analysis is that we combine models of food density dynamics in limited foraging areas and predict that food availability and daily rations should be strongly density dependent leading to density-dependent mortality. Many workers have found that growth and survival are density dependent, particularly for small postsettlement sizes of demersal and reef fish (Lockwood 1980; Ware 1980; Zijlstra et al. 1982; Cook and Armstrong 1986; van der Veer 1986; Peterman and Bradford 1987; Doherty and Williams 1988; Sundby et al. 1989; Daan et al. 1990). However, the mechanism(s) leading to density dependence are rarely indentified (Anderson 1988; Shepherd and Cushing 1990) and the potential importance of these processes for population regulation is rarely specified (Daan et al. 1990; Shepherd and Cushing 1990; Rijnsdorp and van Leeuwen 1992).

It is also not news that natural selection should favor some balancing act in relation to the growth and survival implied by correlation of predation risk and feeding (Abrams 1991; Werner 1992), although the relatively simple balance implied by eq. (10) is perhaps a bit surprising. However, the very existence of a selection balance has at least two profound implications for prediction of the role of ecological factors in recruitment limitation. First, according to the selection argument, it is meaningless to pose simplistic, singe-factor hypothesis like "recruitment is limited by food" or "recruitment is limited by predation" or "recruitment is limited by cannibalism"; the single factor to start with is the selection process itself (i.e., the balancing act). Second, we should distrust trophic predictions of the form "increased predation risk should lead to reduced density and less effect of intraspecific competition." As noted earlier, selection arguments lead to exactly the opposite prediction: increased predation risk should lead to shorter foraging times and smaller foraging volumes V, hence increasing competition and exaggerating density-dependent effects of growth rate.

Moreover, the models we present have serious implications for fisheries enhancement programs aimed at improving early survival. For example, recruitment limitation resulting from predation risk avoidance may explain the apparent failure of hatchery programs to increase abundance of coho (*Oncorhynchus kisutch*) and chinook salmon (*Oncorhynchus tshawytscha*) off the Pacific coast of North America (Walters and Riddell 1986; Emlen et al. 1990) (Fig. 6). During the 1970's, there were various efforts to assess the "carrying capacity" of the North Pacific Ocean for hatchery salmon, based on total food supply and production estimates over large areas (Walters et al. 1978); our general conclusion was that severalfold increases in production should be possible. Instead, what we have seen under increasing hatchery releases is declining marine survival, and little or no increase in large-scale production (small-scale increases from some stocks and hatchery facilities have been mostly balanced by losses in other, usually wild, stocks). While various long-term environmental trends could explain declining trends in marine survival, these survival patterns are certainly consistent with the hypothesis that juvenile coho and chinook are using only a very restricted portion (something like one fifth) of the food production system potentially available to them. Similar conclusions to ours were reached by Salvanes et al. (1992) who developed a model that suggested that juvenile cod enhancement may not always lead to increased production. Increased juvenile stocking may instead result in food and refuge limitation leading to density-dependent growth and survival.

We do not imply from these examples that all enhancement efforts are a waste of time. Predation-related habitat restriction can also explain the dramatic increase in recruitment often seen in marine fish when artifical reefs are provided: these reefs create both "cover" for juvenile fish and feeding volumes V where predators may be attracted by the presence of juveniles.

The arguments presented above also contain the seeds of a possible explanation for the apparent depensatory recruitment patterns (Clark 1974; Murphy 1980) and the delays in stock

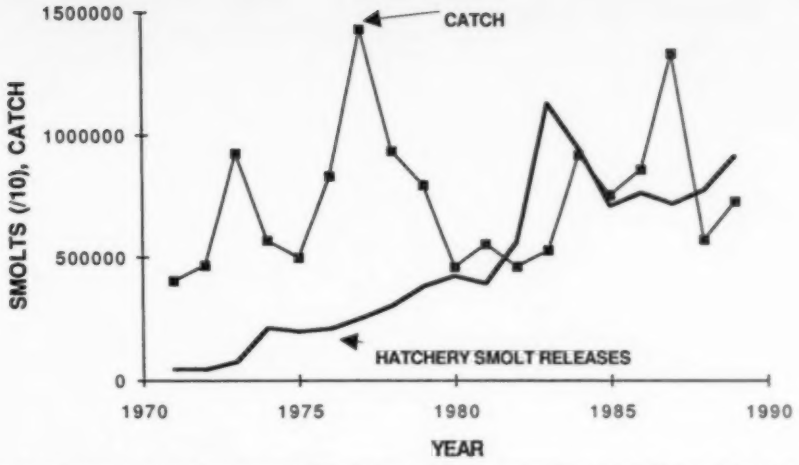

FIG. 6. Increases in coho salmon smolt releases from hatcheries around the Strait of Georgia, B.C., have not been accompanied by consistent increases in sport and commercial catch. Hatchery fish now make up nearly 50% of the catch, so the wild stock contribution has declined substantially. (Source: Department of Fisheries and Oceans catch statistics, Cross et al. 1991.)

recovery that have concerned managers of schooling clupeoid fishes (Wyatt and Larrañeta 1988). If a schooling stock is very low, increases in abundance and shoal/school size may initially result in density-dependent mortality due to individuals spending more time foraging at the school boundaries ("fuzzing" the school boundary), breaking away from schools earlier in the evening, and/or rejoining schools later each morning. This density-dependent effect would tend to stabilize stock size at a low level. But increases in movement and feeding time may also lead to increased probability of localized "range extensions" through formation of new schools and associated colonization of larger areas, with each such relatively rare event leading to a temporary reduction in school size (and mortality rate) and a new, higher apparent "limit" on total recruitment. The apparent limit should increase more rapidly over time as more schools/shoals contribute to the dispersal and colonization process. Under this scenario, oceanographic variations such as changes in current regimes near spawning areas could act as important "triggers" for stock expansion, by altering probabilities of successful dispersal, without being correlated in any obvious way with short-term changes in recruitment rates. Also, any attempt to directly relate recruitment to stock size will show strong and apparently unpredictable patterns of "nonstationarity" (Walters 1987).

A further broad implication of spatial restriction in foraging and associated density-dependent effects is ratio dependence in overall predator–prey relationships between fish and their food organisms, except in cases where foraging volume V is a large proportion of total voume over which food production dynamics take place. (see Ecology Special Feature articles beginning with Berryman 1992, and especially Ginsburg and Akcakaya 1992). Ratio dependence can explain the lack of field evidence in fish–food systems for patterns predicted by classical predator–prey models. In particular, there is little field evidence of predator–prey cycles with periods predicted by classical models, or of the "paradox of enrichment" (destabilization under enrichment or along natural gradients in prey productivity; see McAllister et al. 1972). Fish food organisms are presumably subject to the same selection forces as juvenile fish, trading off behavioral and developmental traits that would maximize their productivity against traits that make them less vulnerable to the fish.

Thus, when we finally see a coevolved food chain, where selection at each link has been influenced by the risks represented by links higher up the chain, we should not expect to see any simple production relationships between successive trophic levels. In other words, selection may well drive ecological efficiencies to far lower values than would be predicted by simple energy flow/loss models. Hence, we should not expect studies of basic aquatic production processes to help much in predicting recruitment to particular fish species.

While evolutionary arguments lead us to be pessimistic about predicting recruitment limits from simple trophic studies, they do not imply that field studies on recruitment mechanisms are a waste of time in general. Based on the models above, we would recommend careful attention in recruitment studies to (1) spatial habitat use by juveniles (assessment of V and variation in prey density within it), (2) a shift in emphasis from study of density-dependent growth and size dependence in mortality to study of how density affects individual time budgets and exposure to predation risk, and (3) study of behavioral and physiological mechanisms for adjusting foraging time and behavior to varying food density and predation risk. In terms of theoretical and modelling research, we recommend the inclusion of time budget optimization calculations in all multispecies modelling efforts.

One useful research approach may be to do careful habitat mapping and juvenile distribution/time budget studies leading to comparative analysis of variation in V among species and locations. This would also lead to estimates of vN/V, a key determinant of impact on local food concentrations within feeding areas.

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Replacement of Sulfur, Carbon, and Nitrogen in Tissue of Growing Broad Whitefish (*Coregonus nasus*) in Response to a Change in Diet Traced by $\delta^{34}S$, $\delta^{13}C$, and $\delta^{15}N$

R.H. Hesslein, K.A. Hallard,[1] and P. Ramlal

Department of Fisheries and Oceans, Central and Arctic Region, Freshwater Institute, 501 University Crescent, Winnipeg, MB R3T 2N6, Canada

Hesslein, R.H., K.A. Hallard, and P. Ramlal. 1993. Replacement of sulfur, carbon, and nitrogen in tissue of growing broad whitefish (*Coregonus nasus*) in response to a change in diet traced by $\delta^{34}S$, $\delta^{13}C$, and $\delta^{15}N$. Can. J. Fish. Aquat. Sci. 50: 2071–2076.

We monitored the change in the isotope composition of sulfur, carbon, and nitrogen in broad whitefish (*Coregonus nasus*) tissues in response to a change in the isotope composition of their food. One of two batches of 2.5-yr-old fish raised in the laboratory were given a new food source with different $\delta^{34}S$, $\delta^{13}C$, and $\delta^{15}N$, which were monitored in muscle and liver tissue for 1 yr. A model including change due to tissue accumulation (growth) and metabolic replacement was developed. For all three isotopes, most of the change could be attributed to growth. Metabolic replacement expressed as a turnover rate was only 0.1–0.2%·d^{-1} and was similar for the three isotopes. Although liver tissue was −4.4 and −4.1‰, respectively, for $\delta^{34}S$ and $\delta^{13}C$ relative to muscle tissue, the response over time to the new food was the same as for muscle. We expect that the complete change in the isotope composition of fish tissue in response to a change in food could take years in slow-growing wild populations. The stable isotope composition would represent a long-term average of the food. In fast-growing fish the rate of change would directly reflect the growth rate.

Nous avons surveillé le changement de la composition isotopique du soufre, du carbone et de l'azote dans des tissus de corégones en réponse à une modification de la composition isotopique de leur nourriture. L'un des deux lots de poissons âgés de 2,5 ans élevés en laboratoire ont reçu une nouvelle nourriture avec des différences dans $\delta^{34}S$, $\delta^{13}C$ et $\delta^{15}N$, qui ont été surveillés pendant 1 an dans les tissus musculaires et hépatiques. Nous avons élaboré un modèle concernant les changements dus à l'accumulation de tissus (croissance) et au remplacement métabolique. Pour les trois isotopes, une bonne partie du changement pouvait être attribuée à la croissance. Le remplacement métabolique, exprimé comme taux de renouvellement, était de seulement 0,1–0,2 %·jour^{-1} et était similaire pour les trois isotopes. Bien qu'on ait pour le tissu hépatique −4,4 et −4,1 ‰ respectivement pour $\delta^{34}S$ et $\delta^{13}C$ par rapport au tissu musculaire, la réaction à la nouvelle nourriture, au bout d'un certain temps, était la même que pour les muscles. Nous pensons que le changement complet de la composition isotopique des tissus de poissons en réaction à un changement dans la nourriture pourrait prendre des années dans les populations sauvages à croissance lente. La composition isotopique stable représenterait une moyenne à long terme de la nourriture. Chez les poissons à croissance rapide, le taux de changement représenterait directement le taux de croissance.

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Over the last few years, our group has used analyses of the stable isotopes of sulfur, carbon, and nitrogen to study the feeding habits of fish with respect to both trophic level and food sources (Hesslein et al. 1991a, 1991b). In the lower Mackenzie River region (N.W.T., Canada), broad whitefish (*Coregonus nasus*) were shown to use food sources with different $\delta^{34}S$ and $\delta^{13}C$. In some cases, this allowed fish to be identified as migratory visitors because their isotope compositions were not consistent with the local food web in the system in which they were caught. In Travaillant Lake, connected to the lower Mackenzie River, broad whitefish had $\delta^{34}S$ spanning a range from 6 ‰ below that of the local food web to nearly (<1‰) the local value. We speculated that some of the fish could have been feeding in Travaillant Lake and their $\delta^{34}S$ was approaching the local value. No data were available to determine how long it would take for the dorsal muscle (the tissue analyzed) to respond isotopically to the new food source.

Studies to determine the time for various organ tissues to change their isotope composition to that of a new food source have been performed on gerbils (Tiezsen et al. 1983), birds (Hobson and Clarke 1992), and shrimp (Parker et al. 1988). In gerbils and birds, organs known to have fast turnover (e.g., liver) changed more rapidly than the slower growing tissues (e.g., muscle). We did not expect that these rates would be applicable to fish but knew that this kind of information would greatly improve interpretation of isotopic data for fish. We therefore present the results of a 1-yr feeding experiment in which we investigated the response of the isotope composition of broad

[1]Present address: Water Quality Branch, Saskatchewan Environment and Public Safety, 3085 Albert Street, Regina, SK S4S 0B1, Canada.

TABLE 1. Characteristics of fish in control and experimental tanks at the beginning of the experiment.

	Control	Experimental
Number of fish	73	73
Total fresh weight (g)	1953	1900
Mean weight (g)	26.8	26.0
SD weight (g)	22.0	21.5
Mean length (mm)	131	129
SD length (mm)	31	32

TABLE 2. Fish food components.

Ingredient	Amount in experimental food ($g \cdot kg\ dry^{-1}$)[a]	Amount in control food ($g \cdot kg\ dry^{-1}$)[b]
Fish meal	350	240
Soybean meal	200	100
Wheat meal	324	283
Cornmeal	0	90
Poultry meal	0	70
Feather meal (hydrolyzed)	0	50
Brewer's yeast	0	50
Vegetable oil (Canola)	100	0
Fish oil	0	80

[a]CTR in Tabachek (1983).
[b]MTT in Tabachek (1983).

whitefish to a change in the isotope composition of sulfur, carbon, and nitrogen in their diet.

Methods

Broad whitefish eggs (seven females) were fertilized with milt from two males of the same catch from the Peel River, N.W.T., Canada, October 25, 1989 (Ratynski and de March 1989). The eggs were transported to the Freshwater Institute in Winnipeg where most hatched during January 1990 and the fish were reared in tanks. The 146 fish we adopted were all about 2.5 yr old (juvenile) at the beginning of the experiment. Due to differences in individual growth, they ranged in size from 51 mm (5.1 g) to 210 mm (325 g). The fish were separated into equal-size control and experimental groups with very similar size distributions (Table 1). Each fish was marked with a binary coded pattern of fin clips (Welch and Mills 1981) to facilitate monitoring of individual growth. Fin erosion occurred from abrasion in the tanks which made reading the code difficult, especially on smaller fish.

Since hatching, all of the fish had been fed the same commercial trout food (Martin Feed Mills Grower Diet) which included marine herring or capelin and grain (mainly soya bean and wheat). A batch of experimental food (Table 2) was prepared similar to the proximate composition of the commercial food (Tabachek 1983). In order to maximize the difference in isotope composition for sulfur, carbon, and nitrogen, we used the muscle of a lake trout (*Salvelinus namaycush*) from Travaillant Lake, N.W.T., Canada, as the fish component in the experimental food. This freshwater fish was the most different isotopically from ocean fish which we could access. The experimental food was pelletized to the same size as the control food and fed to the fish at the same rate. The fish were held at an initial density of $15\ g \cdot L^{-1}$ in 125-L tanks with a continuous flow of dechlorinated water at 10°C. The rate of feeding in each tank was initially $25\ g \cdot d^{-1}$ (approximately 1.25% of the total fresh weight) and was adjusted to maintain a constant proportion to the approximate biomass in each tank as the fish grew and were removed.

At each sampling period, three fish, each from an arbitrary size category, small, medium, and large, were sampled (sacrificed) from each tank. Fish were measured and weighed and when possible, fin clip marks were read. The fish were kept frozen until they could be analyzed. Dorsal muscle tissue was excised from each fish for analysis. Liver samples were analyzed both as whole tissue and after lipids were extracted using chloroform and methanol (Bligh and Dyer 1959).

Stable isotopes were analyzed by the methods described by Hesslein et al. (1989, 1991a). Wet digestion by nitric acid, precipitation of $BaSO_4$, and thermal decomposition to SO_2 was used for $\delta^{34}S$ determinations. Modified Dumas combustion in a sealed tube was used to produce CO_2 for $\delta^{13}C$, and N_2 for $\delta^{15}N$ determinations. Analyses were carried out using a dual-inlet isotope ratio mass spectrometer (VG 602E). All delta values are reported relative to Canyon Diablo ($\delta^{34}S$), PDB ($\delta^{13}C$) and air ($\delta^{15}N$). Precision of the isotopic analyses is 0.3‰ for $\delta^{34}S$, 0.1‰ for $\delta^{13}C$, and 0.3‰ for $\delta^{15}N$.[2]

[2]The stable isotope data for broad whitefish are available, for a nominal fee, from the Depository of Unpublished Data, CISTI, Building M-55, Montreal Rd., Ottawa, ON K1A 0R6, Canada.

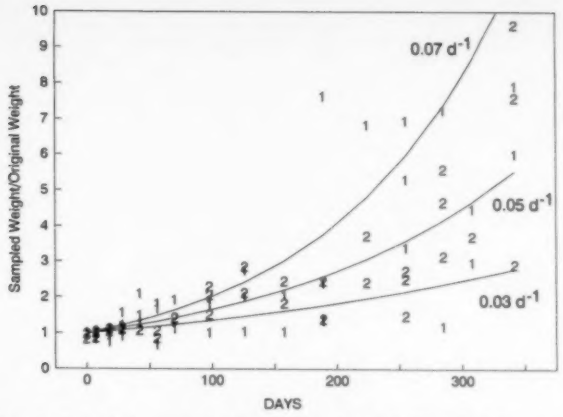

FIG. 1. Growth in control (1) and experimental (2) fish. Lines are exponential models of 0.03, 0.05, and 0.07·d^{-1}.

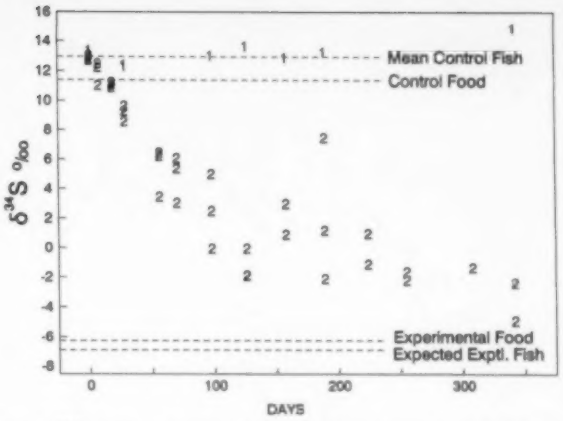

FIG. 2. $\delta^{34}S$ in dorsal muscle of control (1) and experimental (2) fish.

Results

Growth

Because of the difficulties in reading the fin markings of fish, we could not positively identify every fish. Nine of 50 fish caught from the control group and 11 of 50 in the experimental group could not be read. Three additional fish in each group, for which we thought we could read the fins, were significantly shorter when caught than at the start of the experiment. We took this to mean we had misread the marks. This meant we could estimate an average growth rate based on 38 of 50 fish in the control group and 36 of 50 in the experimental group.

There is considerable scatter in the data for the ratio of sampled weight to original weight (Fig. 1). This is probably due to different growth rates among individuals, but some additional misidentification cannot be ruled out. We used a simple exponential growth model for which the best estimate of the growth rate constant for the experimental fish was 0.05·d^{-1}. Average growth of control fish was slightly higher but not significantly different than for experimental fish. Almost all of the estimates on individuals fell in the range of 0.03–0.07·d^{-1} (Fig. 1). Growth is important when considering turnover, as all newly added biomass would have the isotopic signature reflecting that of the new food.

Sulfur

The average $\delta^{34}S$ for the control food was 11.4‰ and for the experimental food −6.3‰, a difference of 17.7‰. The average value of the control fish was 12.9 ± 0.4‰. The 1.5‰ difference between the control food and control fish was probably due to differential assimilation of the food components, as there is no evidence of metabolic fractionation of sulfur isotopes in fish (Hesslein et al. 1991a). Although we have no data for the fish meal used in the control food, oceanic fish typically have a $\delta^{34}S$ of 17–18‰. This suggests that more sulfur was assimilated from the fish component than from the grain component and other ingredients. The lake trout used in the experimental food mix had a $\delta^{34}S$ of −8.3‰. If the same selective assimilation occurred with the experimental food, we expect that the equilibrium value for the experimental broad whitefish should be between −6.3 and −8.3‰. We assumed that the same relationship between bulk food and the fish component existed for the experimental food as for the control food and calculated a best estimate of the expected equilibrium value of −6.9‰ for the experimental fish, or an estimated difference from the control fish of 19.8‰ (Fig. 2).

Carbon

The average $\delta^{13}C$ for the control food was −21.4‰ and for the experimental food was −26.3‰, a difference of 4.9‰. The average value of the control fish was −19.4 ± 0.4‰. As with sulfur, there was a difference in carbon between the control fish and their food of 2.0‰, due to differential assimilation and/or trophic fractionation. Trophic fractionation for carbon has been reported in the range of 0–2‰, (DeNiro and Epstein 1978; Peterson and Fry 1987) although our estimates for natural fish populations are at the lower end (Hesslein et al. 1991a). Differential assimilation is likely to have been a factor because carbon is assimilated from amino acids, lipids, carbohydrates, etc. Our best estimate of the equilibrium expected for fish eating the experimental food was −24.3‰ (2‰ more than the food). This is consistent with the trend exhibited in experimental fish (Fig. 3).

Nitrogen

The $\delta^{15}N$ of the experimental food (9.7‰) was only 1.9‰ different from that of the control food (7.8‰). We could not find a fish source for the food mix that gave a larger difference and had acceptably large differences for $\delta^{34}S$ and $\delta^{13}C$. We had assigned the lowest priority to achieving a difference in $\delta^{15}N$ between experimental and control food because $\delta^{15}N$ has been primarily used as an indicator of trophic structure, not as a food source tracer.

Given both the small difference and the variance, there was low sensitivity for investigating the equilibration of fish to the $\delta^{15}N$ of the new food as compared with $\delta^{34}S$ and $\delta^{13}C$. We were able to see a trophic enrichment of ^{15}N in the control fish versus food of 3.8‰, in the range normally observed (Peterson and Fry 1987; Hesslein et al 1991a). Again, differential assimilation of the food components may have influenced this value. The average $\delta^{15}N$ for control fish was 11.6 ± 0.4‰. The maximum values observed in the experimental fish (at the end of the experiment) were 12.8‰ (Fig. 4), 3.0‰ greater than the experimental food.

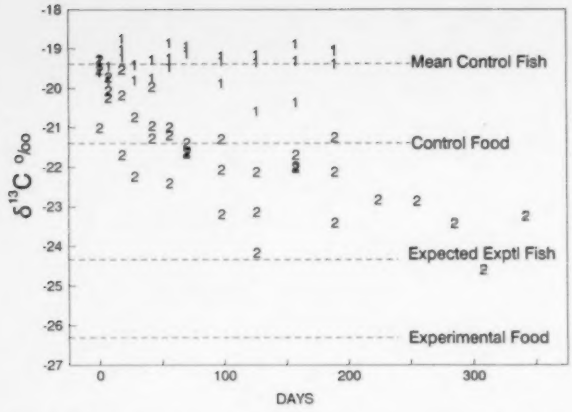

FIG. 3. $\delta^{13}C$ in dorsal muscle of control (1) and experimental (2) fish.

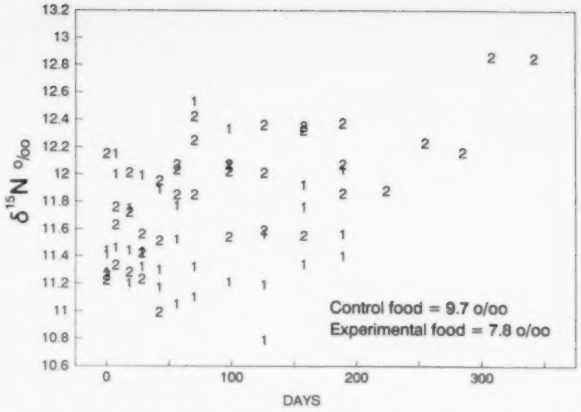

FIG. 4. $\delta^{15}N$ in dorsal muscle of control (1) and experimental (2) fish.

Liver Analyses

$\delta^{34}S$ in liver in four fish in the experimental group sampled over the last 7 mo of the experiment averaged -4.4 ± 0.4‰ relative to muscle. There was no apparent time trend to the difference. On average the $\delta^{13}C$ in liver from experimental fish spanning the entire 12-mo period was -4.1 ± 0.5‰ ($n = 12$) relative to muscle, and there was no significant trend with time ($p > 0.2$). Because lipids have been found to be depleted in ^{13}C relative to muscle from which the lipid had been extracted (DeNiro and Epstein 1977; Tieszen et al. 1983), and because fish liver has a high lipid content, we analyzed some liver samples after lipid extraction. The $\delta^{13}C$ in the livers without lipid averaged 0.7 ± 0.3‰ ($n = 4$) relative to whole livers. Only a small portion of the difference between liver and muscle can be attributed to depleted ^{13}C in lipids. There was no significant difference of $\delta^{15}N$ between liver and muscle (0.5 ± 0.5‰), $n = 12$) and no difference between whole and lipid-extracted liver (0.1 ± 0.2‰, $n = 4$).

Discussion and Modelling

The exponential mass growth model described above represents the growth data fairly well and simplifies the mathematics for further modelling. We do not claim any particular reality for the exponential growth model. In order to predict the isotopic exchange observed in tissue, we have chosen a simple model with two components: growth (tissue addition) and metabolic tissue replacement. The principles of the model are that (1) any body mass added after the change in diet must result in an isotope composition in equilibrium with the new food and (2) a portion of the existing body mass is replaced regularly with or without growth. Other metabolic models for fish (Nordstrom et al. 1976) have included a growth term that has an energy cost (food requirement) associated with growth and a basal metabolic component. We have not used this kind of calorific model because food intake for energy requirements does not necessarily result in tissue replacement.

The δ-value of fish (C) at any time after the switch from the old to the new food, due to growth alone, can be written

$$C = ((W_o \cdot C_o) + ((W - W_o) \cdot C_n)) / W$$

where C_o is the δ-value of fish in equilibrium with the old food, C_n is the δ-value in equilibrium with the new food, W_o is the fish weight (grams) when the food was switched, and W is the fish weight (grams) at the time of measurement. If we use the simple exponential growth model $W = W_o \cdot e^{kt}$ where k is the growth rate (per day) and t is time (days), then

$$dC/dt = -k \cdot (W_o/W) \cdot (C_o - C_n).$$

For tissue replacement alone $dC/dt = -m \cdot (C - C_n)$ where m is the turnover rate constant (per day) Solving the differential equations yields

$$C = C_n + (C_o - C_n) \cdot e^{-(k+m)t}.$$

The growth rate of each fish was calculated when sampled ($\ln(W/W_o)/t = k$). The metabolic turnover constant, m, was the only parameter determined by fitting (least squares) predicted values to the measured data.

For $\delta^{34}S$, $\delta^{13}C$, and $\delta^{15}N$ the model using the growth parameter alone ($m = 0$) explained most of the variance between the observed and modelled isotope composition (Fig. 5). The best fits of m for the model versus measured data (Fig. 6a and 6b) were $0.0013 \cdot d^{-1}$ for $\delta^{34}S$ and $0.0018 \cdot d^{-1}$ for $\delta^{13}C$. We show a value of $0.0018 \cdot d^{-1}$ for $\delta^{15}N$ in Fig. 6c, but because of the poorer sensitivity in the ^{15}N data the "best" fit could not be determined. Because so little of the variance was unaccounted for after growth correction ($r^2 > 0.9$), the sensitivity of the model to m for $\delta^{34}S$ and $\delta^{13}C$ was not sufficient for us to be confident that values of m are different for the different isotopic pairs. In any case, the half-time for metabolic replacement of sulfur, carbon, and nitrogen in the muscle is longer than 1 yr. This indicates that if these rates are typical, the stable isotpes of sulfur, carbon, and nitrogen in the muscle of a slow-growing fish would integrate a very long feeding period.

Since the isotopic analyses of liver showed a constant offset relative to muscle, we must conclude that metabolic replacement in the liver is not much more rapid than in the muscle, unlike previous findings with mammals and birds. The differences in the rates of change of $\delta^{15}N$ were dramatic between gerbil liver ($0.11 \cdot d^{-1}$ and muscle ($0.025 \cdot d^{-1}$) (Tieszen et al. 1983) and quail liver ($0.27 \cdot d^{-1}$) and muscle ($0.056 \cdot d^{-1}$) (Hobson and Clarke 1992). The actual best value for m in the carbon model for liver

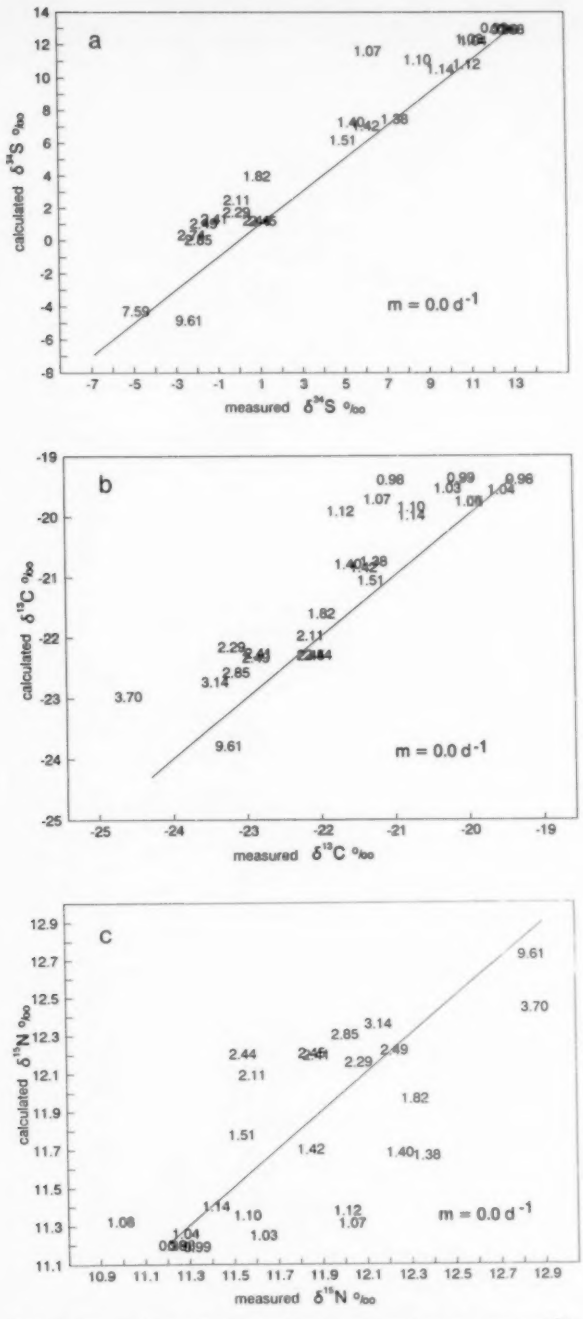

FIG. 5. Comparison of calculated and measured values of (a) $\delta^{34}S$, (b) $\delta^{13}C$, and (d) $\delta^{15}N$ in dorsal muscle of experimental fish using only the growth model (m = 0.0, see text). The model parameters are independent, not fitted to the data.

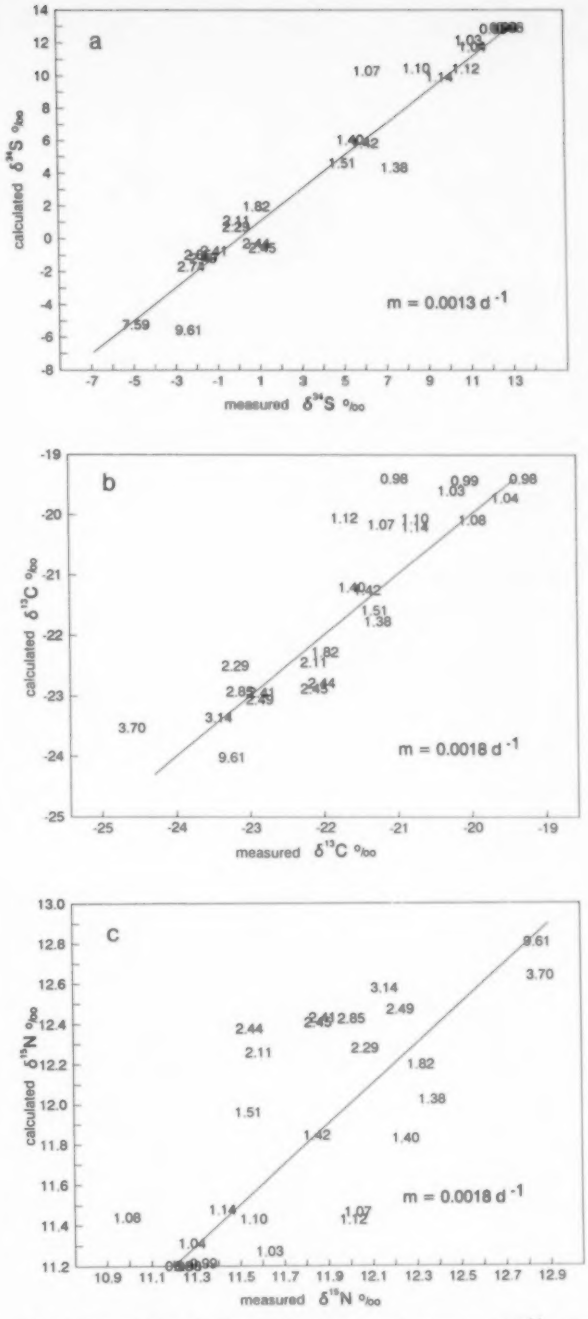

FIG. 6. Comparison of calculated and measured values of (a) $\delta^{34}S$ (m = 0.0013·d^{-1}), (b) $\delta^{13}C$ (m = 0.0018·d^{-1}), and (c) $\delta^{15}N$ (m = 0.0018·d^{-1}) in dorsal muscle of experimental fish using the growth and metabolic replacement model (see text). The growth parameters are independent as in Fig. 5, but m was determined by fitting to the data.

was 0.0025·d^{-1}, slightly, but not significantly, faster than for muscle. This conclusion must be further tempered by the knowledge that the change in the isotopic change in the liver was also primarily driven by growth. The sensitivity in detecting differences in metabolic replacement was not good for muscle and worse for liver because only a subset of the fish were analyzed. It does not appear, however, that the very large differences in replacement observed in warm-blooded animals

(Tieszen et al. 1983; Hobson and Clarke 1992) exist in broad whitefish and perhaps fish in general.

In conclusion, if fish growth is rapid, the change in isotope composition of sulfur, carbon, and nitrogen in response to new food will be dominated by the addition of new tissue. In cases of slow growth, the change in $\delta^{34}S$, $\delta^{13}C$, and $\delta^{15}N$ may be very slow, taking years to reach a new equilibrium. Differences between individuals in groups of slow-growing fish should reflect different food choices because temporal variations should be averaged out. Isotopic differences between individuals in groups of fast-growing fish exposed to a change in the isotope compositions in their diets could be due to different growth rates.

The similarity between liver and muscle analyses in the rate of approach to the new isotopic signal precludes the use of that pair of measurements as a "clock". We had hoped that large differences in the rates at which liver and muscle approached the new food signal would provide a way to estimate the time that a wild population had been consuming a new food source. For fast-growing fish the liver–muscle pair is not a good choice; however, it may still be useful in slowly growing populations. Another tissue pair may prove more useful in identifying fish not in equilibrium with their food source.

Acknowledgements

We thank B. de March for providing the broad whitefish used in the experiment. L. Allard took excellent care of the fish in the Freshwater Institute holding facility. M. Capel fed the fish a number of times over the holiday periods. K. Mills and S. Chalanchuk anaesthetized and marked the fish and helped us to interpret the marks. J. Tabachek taught us how to make fish food.

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Quasi-likelihood Estimation of Marked Fish Recapture

Peter B. Bayley

Center for Aquatic Ecology, Illinois Natural History Survey, 607 E. Peabody Drive, Champaign, IL 61820, USA

Bayley, P.B. 1993. Quasi-likelihood estimation of marked fish recapture. Can. J. Fish. Aquat. Sci. 50: 2077–2085.

In models of the fraction of fish recaptured in field experiments on gear efficiency the binomial error distribution is usually assumed. However, variance in excess of that defined by the error distribution (overdispersion) is typical in fish capture because of heterogeneity among and within groups of individuals and incomplete model specification. Quasi-likelihood offers a parsimonious solution to the typical problem of incomplete definition of an error distribution with discrete responses. An example is given from the recapture of marked fish following rotenone treatment in lake enclosures, in which a generalized linear-logistic model includes an extra-binomial variance as a function of the mean. Estimated standard errors of fitted parameters were two to three times lower in a linear-logistic maximum likelihood model than in the quasi-likelihood model because extra-binomial variation (overdispersion) was ignored in the former model. In a cross-validation trial, 95% confidence intervals included 85% of independent observations with the quasi-likelihood model compared with 69% with the maximum likelihood model.

Dans les modèles de la fraction de poissons recapturés lors des expériences de terrain sur l'efficacité des engins, on pose généralement que la distribution des erreurs est binômiale. Toutefois, la variance qui dépasse celle que définit la distribution des erreurs (surdispersion) est typique dans les captures de poissons à cause de l'hétérogénéité qui règne entre les groupes et au sein des groupes d'individus, et de la spécification incomplète du modèle. La quasi-vraisemblance offre une solution économique au problème typique que pose la définition incomplète d'une distribution des erreurs avec réponses discrètes. Nous donnons pour exemple la recapture de poissons marqués à la suite d'un traitement par la roténone dans des enclos lacustres; un modèle généralisé linéaire-logistique comporte une variance extrabinômiale comme fonction de la moyenne. Les écarts-types estimés des paramètres ajustés étaient deux à trois fois plus bas dans un modèle linéaire-logistique de vraisemblance maximale que dans le modèle de quasi-vraisemblance, parce que la variation extrabinômiale (surdispersion) était ignorée dans le premier modèle. Dans un essai de validation croisée, des intervalles de confiance de 95 % couvraient 85 % des observations indépendantes avec le modèle de quasi-vraisemblance, alors que le modèle de vraisemblance maximale donnait un pourcentage de 69 %.

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Capture–recapture and removal methods for the estimation of fish population size require the prior knowledge or simultaneous estimation of catchability. Exact knowldege of catchability and catches determines the original population size for closed populations (Otis et al. 1978; Schnute 1983), which are those that do not undergo immigrations, emigrations, birth (or recruitment), and mortality (other than that due to removal) during the period of the experiment.

However, catchability is the probability of capture of all individual fish, which is a stochastic variable requiring the assumption of a probability distribution to provide bias-free estimates of the parameters and of their variances. Methods currently in use or recommended (e.g., Otis et al. (1978) for mark–recapture; Zippin (1956, 1958) and Schnute (1983) for removal methods) assume the binomial distribution because the catch is always some proportion of the vulnerable population. Apart from the self-evident assumptions of population closure and a valid marking technique where appropriate, such methods require two principal assumptions.

First, probability of capture is assumed to have a single expected value (or any heterogeneity is known or can be estimated using additional information). Second, probability of capture is an independent, identical, random variable. In other words, each indiviual fish has a binary response of 1 (caught) or 0 (escaped) which is independent of all other individuals, but which has an identical probability distribution. Even though a sum of such binary responses (or Bernoulli trials) may be measured as a binomial response (y_i successes from m_i individuals, or proportion y_i/m_i), the assumption of independence and identical behavior is still necessary for application of the binomial distribution.

Heterogeneity of catchability is now recognized as a major source of bias in population size estimation, and various maximum likelihood approaches have ben suggested (Otis et al. 1978; Schnute 1983). However, in most sampling situations the power to distinguish between models with different catchability assumptions is limited unless explanatory variables can be used to predict those catchabilities (e.g., Pollock et al. 1984). Marginal distributions from the majority of observational studies in nature provide few clues regarding the form of error distributions that are conditional on explanatory variables. The problem is compounded by the effects of unmeasurable or unknown factors as well as by the difficulty of obtaining independent samples.

A crude but not unreasonable regression approach is to transform the variables in an attempt to simultaneously linearize the model in terms of its parameters and homogenize the variance.

The resulting linear analysis lumps all unexplained variance, presuming the errors to be approximately normal and independently distributed. This approach is appropriate for many observational data sets with response variables of unrestricted range. Conversely, for a binomial response (y/m_i) of range (0,1), linear approaches are only valid for large m_i and with few proportions of 0 or 1. For example, the Cox logistic transformation, $Z_i = \log\{y_i + 0.5)/(m_i - y_i + 0.5)\}$, is biased and inappropriate unless all the m_i are fairly large (McCullagh and Nelder 1989).

The generalized linear model (GLM) offers a powerful approach to modeling data when one can presume a distribution from the exponential family (sensu McCullagh and Nelder (1989), which includes the binomial distribution. This was applied to a necessarily complex fish movement – tag recovery model in which the likelihood estimator was assumed to follow a Poisson distribution (Hilborn 1990).

A quasi-likelihood extension of the GLM approach, which accommodates additional variance to that predicted by the theoretical distribution, has not, to my knowledge, received attention in the aquatic sciences, although potential departure from the binomial model has been alluded to in a special context (Schnute 1992). The quasi-likelihood approach has numerous practical applications, one of which is described here with respect to the modeling of catchability data from a set of mark and recapture experiments.

Statistical Approach

A brief overview containing key features of the theory and analysis is provided. Notation style follows Schnute (1983) unless otherwise indicated, and derivations are available in the references.

An approach to the fitting of GLM (Nelder and Wedderburn 1972) requires the definition of three components: the first is stochastic, and the second and third constitute the systematic part of the model.

The first is a probability distribution for a single response variable, y, conditional on a mean value and sometimes other parameters. For the binomial distribution, this is

$$(1)\quad pr[y \mid q, m] = [m!/((m-y)!y!)]\, q^y (1-q)^{m-y}$$

where y is the number of successes out of m trials and q is the mean or expected proportion of successes ($q = E(y)/m$, m = binomial index).

The second is the linear predictor, η, which is determined by k explanatory variables or covariates, x_j (x_0 is unity), and unknown parameters, β_j:

$$(2)\quad \boldsymbol{\eta} = \sum \beta_j x_j = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \ldots + \beta_k x_k = \boldsymbol{\beta' x}\,.$$

The third is a "link-function," $g(q)$, which transforms the mean, q, to the linear predictor, $\boldsymbol{\eta}$. For the binomial response, various functions are appropriate (e.g., probit, complementary log–log), but the logistic is the most widely used:

$$(3)\quad g(q) = \boldsymbol{\eta} = \log_e (q/(1-q)) = \text{logit}\,(q)\,.$$

This linear-logistic relationship maps the proportion of successes, $q(0,1)$, on to a linear combination of explanatory variables, $\boldsymbol{\eta}$ $(-\infty, +\infty)$, using a logistic link function.

In practice, a set of n independent observations, y_i, ($i = 1, 2, \ldots, n$) of the response variable are made with corresponding values of m_i and, optionally, values, x_{ij}, of k explanatory variables x_j. The maximum likelihood (ML) function is derived as follows.

The likelihood function, $L(\beta)$ is the product of probabilities from (1) (ignoring the binomial coefficient, $m!/((m-y)!y!)$, which does not affect the analysis) for n observations:

$$L(\boldsymbol{\beta}) = \prod_{i=1}^{n} q_i^{y_i} (1-q_i)^{m_i - y_i}$$

where $\boldsymbol{\beta}$ is the vector of unknown parameters and q_i is the capture probability for observation i. Solving (3) for q $(=e^{\eta}(1+e^{\eta})^{-1})$ and substituting q for q_i gives

$$L(\boldsymbol{\beta}) = \prod_{i=1}^{n} e^{\eta_i y_i} / (1+e^{\eta_i})^{m_i}\,.$$

The log likelihood, $l(\beta)$, of $L(\beta)$ is

$$l(\boldsymbol{\beta}) = \log L(\boldsymbol{\beta}) = \sum_{i=1}^{n} \left\{\eta_i y_i - m_i \log (1+e^{\eta_i})\right\}$$

which is a more appropriate form for obtaining maximum likelihood estimates by solving first and second-order partial derivatives of $l(\boldsymbol{\beta})$ with respect to the parameters, $\boldsymbol{\beta}$ (Aitken et al. 1989; McCullagh and Nelder 1989).

Maximum likelihood estimators, $\hat{\beta}_j$, of the unknown parameters, β_j, are obtained using an iterative process (iteratively reweighted least squares, Nelder and Wedderburn 1972) which is most conveniently implemented by the GLIM package (Payne 1987). The ML estimates, $\hat{\boldsymbol{\beta}}$, are obtained by solving

$$\boldsymbol{X' W X \hat{\beta}} = \boldsymbol{X' W Z}$$

where $\boldsymbol{X}$ is the matrix ($n \times k$) of observed explanatory variables x_{ij}, $\boldsymbol{W}$ is a diagonal matrix of weights,

$$(4)\quad \boldsymbol{W} = \text{diag}\left[m_i (dq/d\eta_i)^2 / \hat{q}_i (1-\hat{q}_i)\right]$$

$$= \text{diag}\left[m_i \hat{q}_i (1-\hat{q}_i)\right],$$

and $\boldsymbol{Z}$ is the "adjusted dependent variate" estimated by

$$\hat{z}_i = \hat{\eta}_i + (y/m_i - \hat{q}_i)(d\eta/dq_i)\,.$$

The z_i are initially estimated from an approximation of η_i from the data. The $\hat{z}_i$ are then regressed on the x_{ij} with the current weights, $\boldsymbol{W}$, from which new estimates, $\hat{\boldsymbol{\beta}}$, $\hat{\eta}_i$, of $\boldsymbol{\beta}$ and η_i are computed. The regression is repeated with new weights until the estimates $\hat{\boldsymbol{\beta}}$ converge, which usually takes only four or five iterations. The procedure also produces asymptotic estimates of the standard errors of the $\hat{\boldsymbol{\beta}}$ which are accurate for moderately large n.

In this binomial case, the ML estimates of the q_i are influenced by the binomial index, m_i, in the weighting function (4). In a simple example from the model $\eta = \beta_0$, four samples from 32 marked fish of binomial proportions (and $\hat{q}_i$) of 2/8 (0.25), 4/8 (0.50), 4/8 (0.50), and 6/8 (0.75) would produce a mean $\hat{q}$ of 0.50.

However if the 32 individuals produced the same q_i estimates with mixed m_i, such as 1/4 (0.25), 2/4 (0.50), 2/4(0.50), and 15/20 (0.75), the ML estimate of q would be 0.625.

GLIM outputs the model deviance, which is twice the difference between the log-likelihood of the saturated model (i.e., a perfectly fitted model with a parameter for every observation) and that of the proposed model:

$$(5)\quad \text{Model deviance} = -2\left[l\,(\text{model}) - l\,(\text{saturated model})\right].$$

Although models with reduced deviance reflect a closer fit to the data, the model deviance cannot be used directly for a reliable goodness-of-fit test in non-normal models (Aitken et al. 1989; McCullagh and Nelder 1989). However, changes in deviance due to a change in the model, such as subtracting an explanatory variable, result in a maximum likelihood ratio that is asymptotically distributed as χ^2 under the H_0 of no effect (McCullagh and Nelder 1989). For example, if $l(\hat{\boldsymbol{\beta}}_r)$ is the maximum log likelihood of a reduced model with two less parameters than a model with likelihood $l(\hat{\boldsymbol{\beta}})$, then the change in deviance, λ, is a likelihood ratio test statistic which has an asymptotic χ^2 distribution (at 2 df) if the two omitted parameters, β_j, are zero:

$$(6)\quad \text{Change in deviance} = \lambda = -2\left[l\,(\hat{\boldsymbol{\beta}}_r) - l\,(\hat{\boldsymbol{\beta}})\right].$$

Change in deviance is not the same as the difference between two non-nested model deviances because two models will have different saturated model log likelihoods.

The closely related Pearson X^2 statistic is the sum of the squared, standardized residuals $[\,= \Sigma\,(y_i - m_i\hat{q}_i)^2 / \{\,m_i\,\hat{q}_i \times (1-\hat{q}_i)\}]$. X^2 is equal to the degrees of freedom, $n-k$, if the error distribution is binomial, which is presumed in this linear-logistic model. Overdispersion in statistics refers to variance of the response in excess of that defined by a non-normal parametric model. Over- or underdispersed data are indicated when X^2 is more or less, respectively, than the degrees of freedom, suggesting a departure from the binomial model.

Quasi-likelihood Approaches

Overdispersion is much more common than was previously realized and has resulted in numerous proposed solutions (Williams 1975, 1982; Efron 1986; Follmann and Lambert 1986; Moore 1986, 1987; Moore and Tsiatis 1991) and tests (Wilson 1982; Dean 1992) which are mostly in a form that is of limited practical use for the biologist untrained in statistics. The quasi-likelihood approach, which expresses a variance as a function of the mean in order to include a component accounting for overdispersion, was developed by Wedderburn (1974) and extended by Nelder and Pregibon (1978) and others. It is termed quasi-likelihood because the fully specified probability distribution of the random component is unknown, thereby precluding ML procedures to estimate parameters. However, the maximum quasi-likelihood parameter estimates have been shown to have similar properties to their ML counterparts and can produce acceptable results (Wedderburn 1974; McCullagh 1983; Moore 1986; Nelder and Pregibon 1987).

The simplest approach is to scale the estimated binomial variance by a constant estimated by $X^2/(n-p)$ (Wedderburn 1974). A similar procedure was adopted by Pollock et al. (1984). Estimates of standard errors of the parameters are obtained by multiplying the ML estimates by the square root of $X^2/(n-p)$. This approach implies that there is variation among c clusters of constant size, m/c (McCullagh and Nelder 1989), and does not produce well-behaved results if the m_i vary among samples. For example, if some of the independent samples are from single fish ($m_i = 1$), there is no overdispersion whereas overdispersion could result from nonindependent individuals within samples of $m_i > 1$. Biological implications of weighting are developed in the Discussion.

Williams (1982) developed two models that describe extra-binomial variation and also account for variation in m. In the binomial model (1), q, the expected proportion of successes, is constant given $\boldsymbol{\eta}$. Williams (1982) introduced a random variable q' with mean $E(q') = q$ and variance ψ. It can be shown (e.g., Aitken et al. 1989) that

$$(7)\quad \text{Var}(y) = mq\,(1-q) + m\,(m-1)\,\psi$$

where the first term is the binomial variance and the second is the extra-binomial variance. In model II of Williams (1982), $\psi = \phi q_i(1-q_i)$ for each expected value of q_i corresponding to observation i, where ϕ is constant. Therefore, using (7), the binomial variance of the response, y_i, $(m_i q_i(1-q_i))$ is increased by a factor $\{1 + \phi\,(m_i - 1)\}$, and total variance is estimated by

$$(8)\quad \hat{\text{Var}}\,(y_i) = m_i\,\hat{q}_i\,(1-\hat{q}_i)\{1 + \hat{\phi}\,(m_i - 1)\}.$$

Williams (1982) also proposed an alternative model III that added a variance, σ^2, to the logit of q which is the linear component of the model in (2). This is equivalent to assuming $\psi = \sigma^2 q_i^2\,(1-q_i)^2$. Using (7), the binomial variance of the response is consequently increased by a factor $\{1 + \sigma^2\,(m_i - 1) \times q_i(1-q_i)\}$, and total variance is estimated by

$$(9)\quad \hat{\text{Var}}\,(y_i) = m_i\,\hat{q}_i\,(1-\hat{q}_i)\,\{1 + \hat{\sigma}^2\,(m_i - 1)\,\hat{q}_i\,(1-\hat{q}_i)\}.$$

Model III is attractive because random and fixed effects are added on a linear scale analogously to a linear model (Williams 1982) and an approximate confidence interval for logit $(\dot{q})$ can be derived from the estimated variance of individual $\dot{q}_i$ estimates:

$$(10)\quad \hat{\text{Var}}\,\{\text{logit}\,(\hat{\dot{q}}_i)\} \approx \hat{\sigma}^2 + \{m_i\hat{q}_i\,(1-\hat{q}_i)\}^{-1}.$$

The second term in (10) is identical to the maximum likelihood estimate of the variance of logit $(\dot{q}_i)$ for a binomial model by Aitken et al. (1989), who used this to derive more realistic confidence intervals for $\dot{q}$ by back calculation. The estimate of variance in (10) is used here to estimate confidence intervals of the $\dot{q}_i$, but such intervals will probably be underestimates at extreme values of the explanatory variables.

Computationally, model (8) or (9) can be incorporated into the GLIM fitting procedure by applying a prior weight to each observation (i.e. $\boldsymbol{W}$ in (4) is multiplied by the prior weight, which is the inverse of the respective term in curly brackets in (8) or (9)) followed by the automatic iteratively reweighted least squares procedure outlined above to produce an estimate of ϕ or σ^2, respectively. This process is repeated manually until the Pearson X^2 equals the degrees of freedom, $n-k$, resulting in a stable value of ϕ or σ^2 (see Appendix). Convergence to four significant figures was obtained in four or five manual iterations,

TABLE 1. Model fitting with logistic (binomial) and quasi-likelihood assumptions.

Model	Pearson X^2	df	$\hat{\sigma}^2$	Covariates, X_j	$\hat{\beta}_j$	$\widehat{SE}$	Change in deviance[a]	Two-tailed $P(\chi^2)$[c]
(A) Linear-logistic, ML estimates (equation (4))	340	90		Constant	−1.696	0.261		
		1		1/(fish length) (cm^{-1})	−10.25	0.995		
		1		Water temp. (°C)	0.130	0.011		
		1		Enclosure area (ha)	−0.069	0.019		
(B) Quasi-likelihood (weighting based on equation (9))	90	90	0.623	Constant	−1.523	0.522		
		1		1/(fish length) (cm^{-1})	−9.47	2.19	19.6	<0.0001[d]
		1		Water temp. (°C)	0.123	0.022	33.6	<0.0001
		1		Enclosure area (ha)	−0.127	0.066	3.81	0.051
(C) Quasi-likelihood (equation (9)) less 11 points from three enclosures with outlying data	79	79	0.347	Constant	−1.711	0.456	35.6[b]	0.0002
		1		1/(fish length) (cm^{-1})	−11.43	1.89	38.5	<0.0001
		1		Water temp. (°C)	0.149	0.020	63.6	<0.0001
		1		Enclosure area (ha)	−0.163	0.054	9.6	0.0019
(D) Quasi-likelihood (equation (9)), model C data with no covariates	82	82	0.924	Constant	0.312	0.120		

[a]Changes in deviance (maximum likelihood ratios) for model changes show the log likelihood difference (equation (6)) with and without the covariate (explanatory variable), which is testable with χ^2 under the H_0 of $\beta_j = 0$.
[b]The change in deviance of 35.6 reflects the change in model C from model B due to removal of 11 data points from three enclosures (see text).
[c]Tests for coefficients in the logistic model were not made because $X^2 >$ df indicated that the binomial model was not a good fit (see text).
[d]Substitution of fish length produced a smaller deviance of 10.9 ($P = 0.001$).

starting with an initial value of 1 for ϕ or σ^2. GLIM code to solve for ϕ in (8) is provided in Williams (1982), and code to solve for σ^2 in (9) is shown in the Appendix.

Although parametric alternatives, such as the beta-binomial distribution, have been used in ML procedures to account for overdispersion (Williams 1975; Moore 1987), the quasi-likelihood approach does not require assumption of the error distribution in terms of its third and higher moments, has more alternative formulations, and computation is more convenient and faster. However, in common with alternative approaches, it does not guarantee correct specification of the model. Consequently, as indicated in the following example, assessing the model through residual behavior and predictive capability through cross-validation is necessary.

Example: Mark and Recapture of Fish Treated with Rotenone

A series of field experiments in which the numbers of recaptured marked fish following rotenone treatment were counted in 25 lake enclosures was described by Bayley and Austen (1990). A total of 2907 fish were captured by electrofishing, marked, and returned to the enclosures, which contained unmarked fish not used in this study. The choice of enclosures was determined by various management decisions that were independent of factors that directly or indirectly affected capture efficiencies. Fish were carefully caught, handled, measured, marked (using fin-clips), and returned to their typical habitats just prior to the application of the ichthyocide (at 3 ppm of a standard rotenone formulation). This was followed by a standardized fish retrieval process lasting 3 d. The ichthyocide was detoxified using potassium permanganate on the first day.

We attempted an ordinary linear analysis on the capture efficiencies expressed as raw proportions (number of marked fish recaptured/number of marked fish), which indicated strong effects of mean fish length (4–49 cm total length), water temperature (11–28°C), and area of enclosure (0.09–5.09 ha) (Bayley and Austen 1990). However, that model produced invalid predictions of negative values for smaller fish and of proportions greater than 1 for some combinations of the covariates. Also, the residual error from the linear analysis could not be used to infer sensible confidence intervals. Because the purpose of these experiments was to provide an accurate and robust model of the capture efficiency of rotenone, the limitations of the linear analysis called for a more realistic model.

Before model fitting, independence of binomial samples needs to be ascertained in order to make correct inferences from estimated parameters. Each enclosure included various species and sizes of fish. There was considerable overlap in capture efficiencies among species (Bayley and Austen 1990), and no effects could be detected among species or higher taxonomic groups that contained similar-sized fish. Consequently, all species were combined and pooled within length groups from each enclosure. Choice of length groups of marked fish affected the degree of independence among the resulting binomial samples. Combining data to produce a single measurement per enclosure collapsed the model over fish length, which is a covariate known to influence efficiency, and would therefore introduce heterogeneity into the binomial model. Randomly selecting subsets of fish of restricted length range from each enclosure experiment would ignore most of the data and result in a very approximate model. Cutting the data into small size groups would be expected to decrease independence because schools or cohorts of similar-sized fish would be expected to respond similarly. When using samples from 5-cm length intervals that were ordered by fish length and proximate enclosures, standardized residuals resulting from a quasi-likelihood logistic fit failed the Wald–Wolfowitz runs test (SYSTAT 1992)

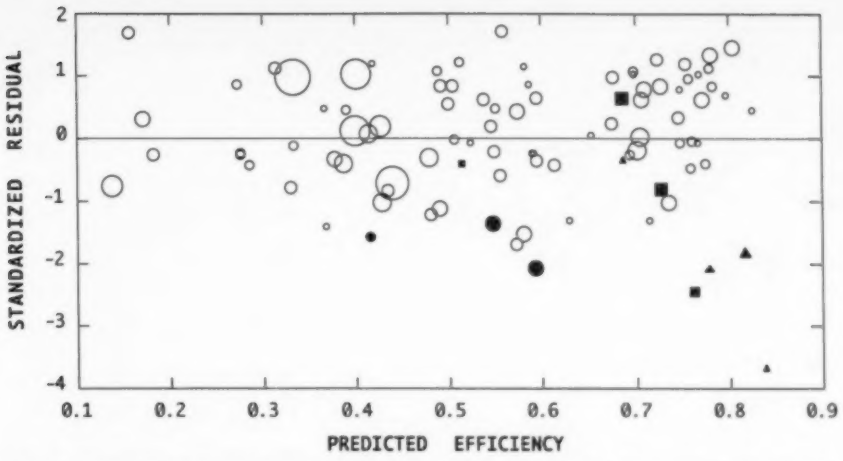

FIG. 1. Standardized residuals from model B (Table 1). Area of symbols is proportional to the binomial index, m_i, of each sample (range 1–301). Eleven solid symbols represent samples from different size classes from three enclosures (circles, squares, triangles) that included outliers (see text), and the open circles represent samples from the remaining 22 enclosures.

about zero ($P = 0.001$ from 214 residuals). Thus, I used a coarser grouping of four intervals (2 = <10 cm, 10 = <20 cm, 20 = <35 cm, 35 = <70 cm total length) that produced residuals lacking indication of a significantly nonrandom sequence of data (Wald–Wolfowitz test: $P = 0.15$ from 94 residuals).

This level of independence among binomial samples does not ensure that the responses of individual fish are independent at the binary level. The runs test of narrow length intervals described above and the following analysis suggest that responses of individual fish are not independent.

Model Fitting

A linear-logistic fit to all the data assuming the binomial model is shown in Table 1, model A, with the covariates of 1/(mean fish length), water temperature, and enclosure area. When mean fish length was used instead of its inverse, a smaller change of deviance (10.9 compared with 19.6, Table 1) resulted when the covariate was removed from the model; therefore, its inverse was preferred. Two other, uncorrelated covariates that were considered likely candidates, mean depth and water transparency, were not significant ($P > 0.1$). Estimated standard errors were small compared with their respective estimated coefficients, but the Pearson X^2 was much larger than the degrees of freedom, indicating overdispersion relative to the binomial model. Consequently, quasi-likelihood model III (9) was fitted (Table 1, model B) which produced standard errors of parameter estimates that were two to three times larger because of the effect of extra-binomial variance. Including all interactions (product terms) of the explanatory variables only reduced the deviance (6) by a nonsignificant 1.81 (4 df). Therefore, interaction terms were not indicated for this model.

Quasi-likelihood model II (8) produced very similar results to model III (9), with a model deviance (5) differing by 0.5% and maximum changes among the parameter and standard error estimates of 3 and 3.5% respectively. Model III is preferred because of the simpler procedure for estimating approximate confidence intervals (see (10) and Cross-validation).

Standardized residuals from the model III fit, using the more accurate formulations of Williams (1982) rather than Pearson's residuals, suggested some low values resulting from three enclosures (Fig. 1). Two of these enclosures had much higher escapement into neighboring enclosures that the other 20 that could be monitored, while the third did not have a neighbor (Bayley and Austen 1990). Removing all data (11 values) from these three enclosures with outliers (Fig. 1) reduced the deviance by a highly significant 35.6 ($P = 0.0002$; 11df). The resulting model C (Table 1) is shown with measured efficiencies versus fish length for subsets of covariates in Fig. 2A and 2B, and residual efficiency versus predicted efficiency for all 83 data points is shown in Fig. 2C. The decision to retain or remove enclosure data containing outliers depends on the application of the model (see Discussion).

Model C (Table 1) produced residuals with no trend with respect to the estimated efficiencies (Fig. 2C), or with any of the covariates. The significance of enclosure area was much more convincing than in model B, and the extra-binomial variance estimate, σ^2, was almost halved. The overall improvement due to the covariates can be observed by comparing the quasi-likelihood fit to the constant model, $\eta = \beta_0$, in which $\hat{\sigma}^2$ was 2.6 times larger (Table 1, model D).

The estimate of extra-binomial variance of 0.347 in model C may still be partly attributable to unknown factors in each enclosure. It was not feasible to design an experiment that statistically blocked enclosures while simultaneously accounting for the enclosure covariates, temperature and area. However, the standardized residuals closely approximated a normal distribution (although there was no theoretical reason to indicate this), and, consequently, a one-way ANOVA of the residuals by coves was performed. This analysis indicated that low significance ($P = 0.084$; 1,21 df) could be attached to residual cove effects.

Cross-validation

Random numbers were assigned to each of the 83 observations included in model C (Table 1), and model III was fitted to the 44 observations having random numbers between 0 and 0.5. Predictions from this model were compared with the independent values from the remaining 39 observations (Fig. 3).

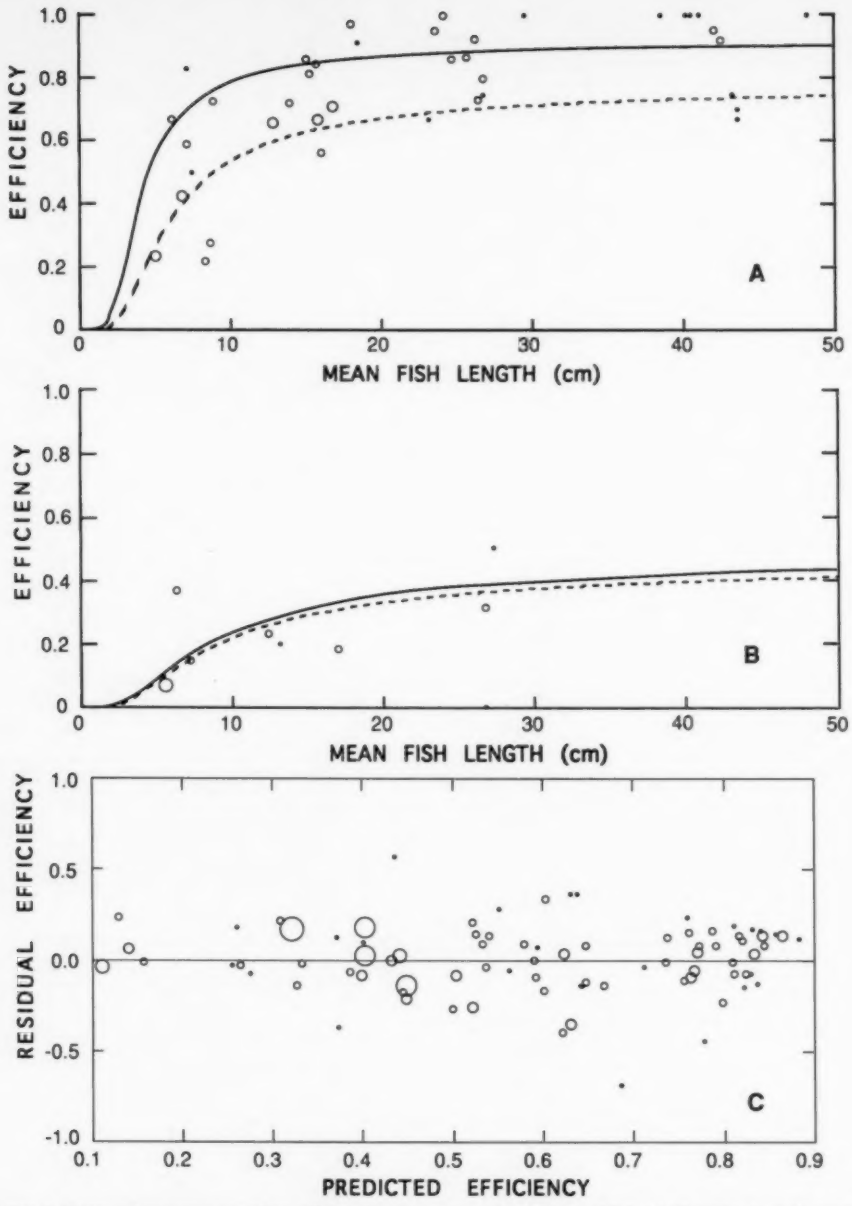

FIG. 2. Observed rotenone sampling efficiencies versus mean total length of fish. (A) Samples in enclosures of 21–28°C and 0.11–0.82 ha; (B) samples in enclosures of 11–11.5°C and 0.15–0.27 ha. Curves show the predicted values from model C (Table 1) corresponding to the highest (solid line) and lowest (broken line) combined effects of temperature and area. (C) Residual efficiencies versus predicted efficiencies of all 83 values from the 22 enclosures used in model C. Area of symbols is proportional to the binomial index, m_i, of each sample (range 1–301).

Approximate 95% confidence intervals were estimated for each predicted value using the square root of (10) as a standard error estimate and assuming a normal distribution on the logit scale. Upper and lower limits were transformed to the q-scale using (3) and compared with the independently measured values (ranges for $m_i < 25$ were omitted from Fig. 3 for clarity). Basing confidence limits on the logistic scale provides more realistic estimates of q within the proportion range (0,1) (Aitken et al. 1989), but the back calculation from the logit cannot include 0 or 1 in the range. Excluding the six observed efficiencies with values 0 or 1, 85% of the remaining 33 observations were within the 95% confidence values. The 85% value would have been higher if the estimated extra-binomial variance ($\hat{\sigma}^2 = 0.248$) from the random 44 observations had not underestimated the

corresponding value ($\hat{\sigma}^2 = 0.347$) estimated from all 83 values in model C (Table 1) by nearly a third. An ordinary logistic fit to the 44 observations produced confidence intervals (using (10) with zero extra-binomial variance) that only included 69% of the same 33 observations.

Discussion

An essential difference between generalized models based on discrete distributions of the response variable, such as the binomial or Poisson, and ordinary linear models assuming a constant variance is that the latter include all unexplained variance in an error term. By contrast, typical discrete-response models contain the assumption of a proportion or count which is determined by the linear (2) and link (3) parts, with variance fully explained by the probability density function (e.g., (1)). Any additional variance in that response should be explicitly modeled as an extension to the model.

If the probability of fish capture was like the repeated tossing of a coin, a binomial variance would be expected. However, the observed variance is expected to exceed the binomial because of three factors: (i) the systematic part of the model ((2) and (3)) describing the capture efficiency through explanatory variables and the link function may be incomplete or incorrect, (ii) fish in a school or sharing a habitat do not behave independently (clustering), and (iii) unlike a coin, individual fish behave differently from one another (i.e., they do not have identical probability distributions).

In this example, the term "behave" can apply to the response of a dead fish during the capture process (e.g., differing propensity to rise to the surface and be observed in the case of rotenone) as well as of live fish (e.g., differing ability to avoid or resist local concentrations of rotenone). Any of these factors results in an expected response that is constant among individuals. I would expect at least as much variation in response to other capture methods that do not kill the fish. Capture efficiency data from six distinct stream fish sampling methods indicated similar or greater extra-binomial variances (Bayley and Dowling 1990) compared with this study.

With respect to factor (i), the ANOVA on residuals suggests that additional enclosure attributes were not significant. However, subtle effects of unmeasured habitats within enclosures may have occurred, such as submerged structures that may have selectively reduced the retrieval of some fish groups. Factor (i) may also account for additional variance if the link function is not optimal, but in this data set the probit link gave similar results, which is often the case unless very large samples which better define the tails of the distribution are available.

If a good systematic description is obtained in the model, factors (ii) and (iii) will still be expected to influence the variance of the capture efficiency, producing an extra-binomial variance that should be estimated even for the simplest fishing methods. Underdispersion, which could result from compensatory effects among the behavioral responses of individual fish, is considered unlikely. I consider that factor (ii), resulting from group responses (termed clustering effect among some statisticians), to be the principal cause of overdispersion for the majority of fish capture methods. If groups of fish were to act as "single fish" and be counted as such, the binomial variance would be much higher. As a hypothetical example, assume that the data in model C (Table 1) represented a single expected efficiency of $q = 0.5$ (i.e. $\eta = \beta_0$) with the same estimated extra-binomial variance of

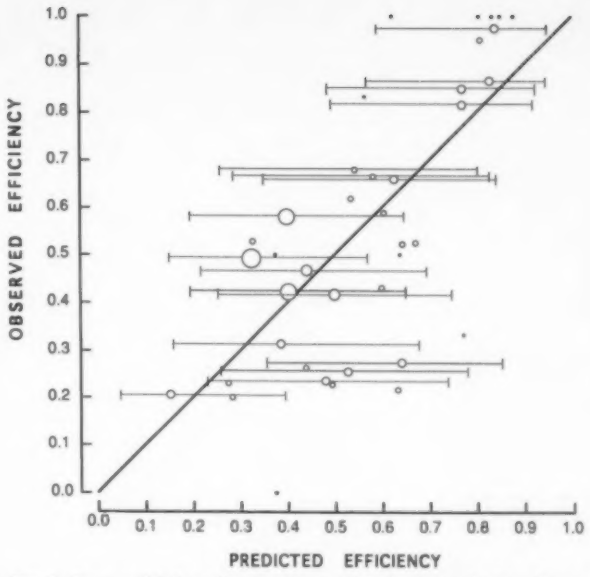

FIG. 3. Cross-validation plot showing predicted efficiencies based on a quasi-likelihood (equation (9)) fit of 44 randomly selected points and using the covariate values corresponding to the remaining 39 observations whose values are indicated by the ordinate scale. Approximate 95% confidence intervals (see (10) and text under Cross-validation) are shown for samples of >25 individuals. Area of symbols is proportional to the binomial index, m_i, of each sample (range 1–301).

0.347. The total variance on the logit scale (equation (10)) would be 0.347 plus the binomial variance (0.133) corresponding to $q = 0.5$ and the average m_i of about 30. This total variance (0.48) would be equivalent to the binomial variance produced by an average m_i of about eight fish. Therefore, about one quarter (8/30) of the number of fish marked would produce this variance. In other words, independent, random "schools" of about four fish, with each fish behaving exactly the same in each school, would produce a binomial variance equal to the observed variance, even though each school has the same expected catchability.

In practice, there will probably be a mixture of this group (clustering) effect (factor (ii)), with some individual variation within groups (factor (iii)) and some dependency of individuals or groups on unmeasured external variables (factor (i)). The prior weights in (8) and (9) (i.e., the inverse of the terms in curly brackets) can be perceived to roughly simulate the combination of clustering and individual effects by mitigating the proportional effect of m_i in the standard weight used in fitting the logistic model (4). In other words, when groups comprising m_i similar-sized fish are larger, clustering and individual effects will increase, but cluster size will increase by some fractional power of m_i. In the extreme case of cluster size = m_i with no individual heterogeneity, samples should be regarded as binary with all $m_i = 1$.

Only a method that captures fish singly on the basis of independent, random samples (which would require a prohibitively high number of samples for inferences on catchability or population size) would be expected to conform to the independence condition of the probability model. However, the efforts described above to obtain independent binomial samples

are still important, even if fish are not independent within those samples when $m_i > 1$, because lack of independence among samples will invalidate the testing of regression coefficients in (2).

Treatment of outliers depends on the application of the model. One purpose was to provide a robust model to estimate abundance of all fish in those enclosures, in order to determine the population that was vulnerable to other fishing methods. In this case, removal of outliers is acceptable to predict populations in the 22 enclosures. Populations in the remaining three could be estimated by including dummy variables for each of those enclosures. Conversely, predicting populations in new enclosures based on rotenone capture of unmarked fish would necessitate the less precise model B that includes the outliers. Alternatively, if marked fish are used in each new enclosure, model C can be tested and updated with the new information to produce a more precise estimate (Bayley and Austen 1990).

Work on statistical theory, simulation, and applied examples is needed before the generality of various quasi-likelihood functions can be ascertained and software developed to avoid the laborious data manipulation and graphical exercises currently necessary to determine the best model for each data set. Better approximations of confidence intervals might be obtained by deriving estimation methods for prediction intervals for the response variable (catchability) for models with more than one covariate. However, the practical difficulties of evaluating the sources of additional variance of fish catchability, even if fish are individually marked, will limit the appropriate application of theoretical distributions, and the use of cross-validation and other diagnostic tools on parsimonious models should dictate applications in the foreseeable future.

In conclusion, fitting of a discrete model with a predetermined distribution that does not allow for additional variation runs the risk of underestimating the confidence intervals of predicted catchabilities and underestimating standard errors of explanatory variables. Most mark–recapture and removal models used to estimate populations fall into this category. Conversely, I predict that modeling for additional variance, such as in simple quasi-likelihood extensions, will be more appropriate even if the model is incorrectly specified. This risk also applies to maximum likelihood models simulating field observations in fisheries because assumptions required by the theoretical error distributions that account for additional variance are so difficult to test. Incorrect or incomplete specification is acceptable for most applications if the residuals and predictability can be shown to be well behaved.

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Appendix: GLIM Code for Estimating Extra-binomial Variance, σ^2

This appendix briefly describes the process for obtaining estimates for (9) (model III of Williams 1982) after the data have been loaded into GLIM. Notation for standard GLIM variables and other commands (beginning with $) are described in the package manual (Payne 1987) and the other variables in Williams (1982).

The model is set up with the commands $YVAR Y $ERROR B M $WEIGHT W$ (where Y is the response, y_i, B is for the binomial model with binomial index, M = m_i, and the logit link function as the default; and W are initial weights set to 1 for all observations).

The initial fit of the parameters is obtained by $FIT X1+ X2+. . .$ (in this example, X1 = reciprocal of fish length, X2 = temperature, and X3 = enclosure area). Then the following macro MOD3 is executed ($USE MOD3) to obtain the first estimate of σ^2 (%P) and the prior weights (W) used in the next manual iteration:

```
$MACMOD3!
$EXTRACT %VL$CALC WVQ = %PW*%WT*%VL!
$CALC %N=%CU(%PW*(1-WVQ))!
$CALC%O= %CU(%WT*(1-1/%BD)*%PW*(1-WVQ))!
$CALC%P=(%X2-%N)/%O:
    W=CWT/(1+%P*(1-1/%BD)*%WT) $WEIGHT W !
```

Then the $FIT command is repeated ($FIT .$ includes the initially defined explanatory variable list) to obtain new estimates of the regression coefficients, followed by $LO %P$ to inspect the latest estimate of σ^2. The process is repeated starting with execution of MOD3 followed by the $FIT and $LO commands until the successive σ^2 estimates converge (which reflects the equality of Pearson X^2 and the degrees of freedom). Fitted parameter and standard error estimates are displayed by $DIS E$.

Ballast Water Exchange as a Means of Controlling Dispersal of Freshwater Organisms by Ships

A. Locke[1]

Department of Zoology, Erindale College, University of Toronto, Mississauga, ON L5L 1C6, Canada

D.M. Reid and H.C. van Leeuwen

Department of Zoology, Erindale College, University of Toronto, Mississauga, ON L5L 1C6, Canada

and

Maritime Studies Program, Williams College - Mystic Seaport, Mystic, CT 06355, USA

W.G. Sprules

Department of Zoology, Erindale College, University of Toronto, Mississauga, ON L5L 1C6, Canada

and J.T. Carlton

Maritime Studies Program, Williams College - Mystic Seaport, Mystic, CT 06355, USA

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During May–December 1990 and March–May 1991, 546 foreign ocean-going vessels entered the Laurentian Great Lakes and upper St. Lawrence River, areas protected by the Great Lakes Ballast Water Control Guidelines. Between 88 and 94% of the vessels exchanged their ballast water with seawater as required by the guidelines. Living representatives of 11 invertebrate phyla were sampled from ballast tanks. Between 14 and 33% of ships that exchanged freshwater ballast in midocean carried living freshwater-tolerant zooplankton at the time of entry to the Seaway, although these included many taxa already found in the Great Lakes. Four freshwater-tolerant zooplankton species that were identified as living specimens in ballast water have apparently not been recorded from the Great Lakes. Voluntary ballast water controls reduced but did not eliminate the risk of species invasion, since some ships did not comply with the guidelines, and even ships that did exchange ballast water could introduce viable freshwater-tolerant organisms into the Great Lakes. About half of the ballast water carried into the Seaway by ocean-going vessels and lakers each year originates from the St. Lawrence River, portions of which are not yet protected by any ballast controls.

De mai à décembre 1990 et de mars à mai 1991, 546 transatlantiques étrangers sont entrés dans les Grands Lacs Laurentiens et le cours supérieur du Saint-Laurent, zones protégées par les Lignes directrices visant le contrôle de l'eau de lest dans les Grands Lacs. Entre 88 et 94 % des bateaux avaient remplacé leur eau de lest par de l'eau de mer, comme l'exigent les lignes directrices. Des représentants vivants de 11 phylums d'invertébrés ont été échantillonnés dans les ballasts. Entre 14 et 33 % des navires qui avaient remplacé l'eau douce de leur réservoir au milieu de l'océan transportaient du zooplancton vivant tolérant à l'eau douce au moment de leur entrée dans la voie maritime, mais on y retrouvait de nombreux taxons qui existaient déjà dans les Grands Lacs. Quatre espèces zooplanctoniques tolérantes à l'eau douce, identifiées à l'état vivant dans l'eau de lest, n'avaient apparemment pas été signalées dans les Grands Lacs. Les contrôles volontaires de l'eau de lest ont réduit mais n'ont pas éliminé le risque d'invasion par certaines espèces, car certains navires ne respectaient pas les lignes directrices, et même ceux qui avaient changé leur eau de lest pouvaient introduire dans les Grands Lacs des organismes viables tolérants à l'eau douce. Environ la moitié de l'eau de lest amenée dans la voie maritime par les transatlantiques et les cargos des Grands Lacs provient du Saint-Laurent, dont certaines portions ne sont pas encore protégées par des contrôles de l'eau de lest.

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A large volume of water, much of it originating from harbours or coastal waters, has been moved about the world as ship's ballast since the use of water ballast commenced in the mid-1800s (Carlton 1985). Ballast requirements and capacities vary with ship design, but modern ships on transoceanic voyages may carry as much as 25–35% of their deadweight tonnage as ballast water, some vessels carrying in excess of 150 000 tonnes (Schormann et al. 1990). Typically, water is pumped into ballast tanks or empty cargo tanks as the ship leaves port and discharged when it takes on cargo at another port. Living animals and plants suspended in the water column adjacent to the ship may enter the ballast tank if they can pass

[1]Present address: Department of Fisheries and Oceans, Marine and Anadromous Fish Division, Gulf Fisheries Centre, P.O. Box 5030, Moncton, NB E1C 9B6, Canada.

through the intake gratings (usually 1–1.5 cm in diameter, but sometimes enlarged by corrosion; Carlton 1985) and survive the impeller pump blades. The ballast tank environment is dark, sometimes oxygen-poor, and usually with limited food resources, but some organisms usually survive the voyage (Medcof 1975; Carlton 1985; Williams et al. 1988).

The role of ballast water in dispersing biota was largely neglected for almost a century. Probably the first collection of living organisms from a ballast tank at the end of a voyage occurred in 1973 (Medcof 1975). Appropriately, this early research took place in Australia, which has now documented the establishment of at least 14 species known with reasonable certainty to have arrived in ballast water (Jones 1991). Worldwide, at least 100 species that were probably transported in ballast water have successfully invaded new environments, including phytoplankton, siphonophores, gastropods, bivalves, cyclopoid copepods, calanoid copepods, cladocerans, amphipods, mysids, shrimps, crabs, and fishes (Carlton 1985; Jones 1991; Mills et al. 1991). In all, 12 phyla of animals and plants are known to have survived transport in ballast water, in some cases for as long as 95 d (Carlton 1985).

Ballast water carried by ocean-going vessels has been identified as a vector for introduction of a variety of freshwater organisms into the Great Lakes. The possibility of such introductions has been acknowledged for more than a decade (e.g., Howarth 1981). Between 1984 and 1990, at least six European species (the cladoceran *Bythotrephes cederstroemi*, two species of zebra mussel (*Dreissena* spp.), and three fishes, the ruffe *Gymnocephalus cernuus* and the gobies *Neogobius melanostomus* and *Proterorhinus mormoratus*), which were probably introduced in ballast water, established populations (Mills et al. 1991). Recognition of potential economic and environmental repercussions of these species introductions resulted in the development of voluntary ballast water exchange procedures, the "Great Lakes Ballast Water Control Guidelines" (GLBWCG), intended to limit unplanned introductions of nonnative species into the Great Lakes. The GLBWCG require that ships destined for the St. Lawrence Seaway and upper St. Lawrence River replace all ballast taken on in fresh or coastal marine waters with midocean water.

The purpose of this paper is to examine the effectiveness of these ballast control procedures. We assess rates of voluntary compliance with the GLBWCG and determine the effectiveness of open-ocean ballast water exchange by foreign ocean-going vessels entering the St. Lawrence Seaway and upper St. Lawrence River in 1990 and 1991. Compliance rates were calculated using information obtained from vessel operators, salinity measurements, and zooplankton collections. Effectiveness of ballast water exchange, i.e., its ability to eliminate living individuals of freshwater-tolerant taxa, was determined for zooplankton. Our objective was to provide empirical evidence to evaluate the efficacy of current ballast control procedures in protecting the Great Lakes. The findings are intended to supersede the preliminary results of Locke et al. (1991), and indeed contradict those results in some respects.

Materials and Methods

Data Collection

From May 29 to December 10, 1990, we collected information on the origin, exchange history, and quantity of ballast water carried by 336 upbound ocean-going vessels entering the St. Lawrence Seaway (the area protected by the 1990 GLBWCG). From March 30 to May 28, 1991, we collected information on an additional 210 vessels upbound beyond Quebec City (the area protected by the 1991 GLBWCG). Information was supplied by vessel operators and was obtained through the Canadian Coast Guard, the St. Lawrence Seaway Authority, and pilotage sources (Locke et al. 1991). Only vessels of foreign registry were used in this study because more information was available for them than for Canadian- or American-registered vessels, which have fewer interactions with Canadian regulatory agencies prior to entering the Seaway. In addition, very few Canadian or American vessels carried ballast water from ports outside the Great Lakes drainage.

We supplemented these data with interviews of vessel operators on 134 vessels transiting the Seaway locks in Montreal or docked in Montreal Harbour. On 86 of these vessels (Locke et al. 1991, 1992), temperature and salinity measurements and zooplankton samples were obtained from one or more ballast tanks. Temperature and salinity were measured using a mercury thermometer (Ertco K-5352) and refractometer (Atago S-10, NSG Precision Cells Inc., Farmingdale, N.Y.), respectively. One to five zooplankton samples were collected on each vessel either by pumping water from a deck tap through a plankton net or by removing a hatch and towing the net vertically through the water in the ballast tank from as close to the bottom of the tank as possible. Most tanks were sampled using both a 41-μm-mesh (13-cm-diameter) and a 110-μm-mesh (13-, or 30-cm-diameter) plankton net. Volume of water sampled (corrected for net efficiency) varied between 24 and 6000 L (rarely less than 200 L). Zooplankton were rinsed from the nets and returned unpreserved to the laboratory for microscopic examination within 2 h of collection in order to determine which zooplankton were alive. Zooplankton were then treated with a "vital stain" (bacto-neutral red) specific to active mitochondria (Summer 1969) and preserved in 4% sucrose–formalin mixture buffered with 10% magnesium carbonate (Lind 1979). Preserved specimens were enumerated and identified to species where possible.

The GLBWCG and Rates of Compliance

The rationale of the GLBWCG is that the organisms that exist in midocean are generally less tolerant of low or fluctuating salinities than those from coastal waters and consequently would be less likely to survive an accidental introduction into the Great Lakes. The original guidelines, put into effect on May 1, 1989, requested that ships upbound in the St. Lawrence Seaway carry only ballast water that was taken on in ocean depths greater than 2000 m. Ships unable to exchange ballast water in midocean (e.g., because of dangerous weather conditions, or because the vessel had not left the North American continental shelf) were requested to exchange ballast water within the Laurentian Channel, east of 64°W longitude, in water depths exceeding 340 m. Changes made to the guidelines since their introduction have increasingly tightened controls on ballast water. An amendment in April 1990 restricted ballast water exchanges in the Laurentian Channel to the area between 61 and 63°W and to water depths exceeding 300 m. Another amendment in February 1991 expanded the guidelines to include vessels proceeding upbound beyond Quebec City, the lower limit of freshwater in the St. Lawrence estuary (El-Sabh 1979). In March 1991 the Canadian and U.S. Coast Guards issued joint voluntary guidelines reaffirming the Canadian guidelines then in effect. It is

TABLE 1. Sources of ballast water carried by foreign ocean-going vessels. One vessel may carry water from one or more sources.

Source of ballast water	Percentage of vessels carrying some ballast water from source	
	Before exchange	After exchange
Atlantic Ocean	15	60
Laurentian Channel	3	14
St. Lawrence/Saguenay rivers	2	11
Montreal Harbour	3	11
Other North American ports	13	5
Mediterranean/Black Sea	8	2
Baltic/North Sea, English Channel	7	2
European ports	47	5
Caribbean Sea	2	4
Central/South American ports	3	<1
Pacific Ocean	3	2
Indian Ocean	0	<1
Asian ports	3	<1
Sea of Japan	<1	0
African ports	5	<1
Australian ports	<1	0
Number of vessels	104	259

intended that ballast water exchange will become a legal requirement for vessels transiting the American portion of the St. Lawrence Seaway.

Because the areas protected by GLBWCG differed between the 1990 and 1991 field seasons, we calculated compliance in 1990 using only vessels that entered the St. Lawrence Seaway, and in 1991 using all vessels that reached Montreal harbour even if they did not enter the Seaway. In conformance with current Canadian Coast Guard practices, we considered vessels to be in compliance with the GLBWCG if they: (1) carried no ballast water, (2) carried only residual ballast water that could not be completely expelled, (3) carried permanent ballast water, (4) carried ballast water that was not intended to be discharged in the Great Lakes (whether or not this water had been exchanged according to the GLBWCG), or (5) carried only ballast water that had been exchanged offshore or in the Laurentian Channel as requested by the GLBWCG. The April 1990 amendment of the limits of the Laurentian Channel "alternative exchange area" was not always communicated to incoming vessels (D.M. Reid and H.C. van Leeuwen, personal observation), so in 1990, we used the original limits of this area (Laurentian Channel east of 64°W longitude, with water depth exceeding 340 m). In 1991, we used the April 1990 amendment to delimit the alternative exchange area.

We calculated compliance rates in two ways: (1) including all vessels even if they reported no ballast water and (2) including only vessels known to be carrying ballast water.

Determining Effectiveness of Midocean Exchange

We used zooplankton as a model for aquatic organisms that might be transported in ballast tanks. Zooplankton are taxonomically better known than phytoplankton and more easily categorized into freshwater, coastal, and oceanic species. They are more easily sampled from ballast tanks than fishes because zooplankton are usually more abundant than fishes and less able to avoid capture by a net.

Effectiveness of midocean exchange in eliminating living individuals of freshwater-tolerant zooplankton taxa was determined for vessels that had originated from a fresh- or brackish-water port. Exchange was considered to be effective in vessels that originated from a fresh- or brackish-water port, reported exchange of ballast water in compliance with GLBWCG, and did not carry living individuals of freshwater-tolerant taxa at the time of sampling. Zooplankton were categorized according to their ability to survive in freshwaters as described in the literature. Some of the invertebrates we included in our analyses are not generally considered to be planktonic, but were collected from the water column inside one or more ballast tanks. Among the taxa we collected in ballast water, we considered all Oligochaeta and selected Rotifera (*Asplanchna, Brachionus, Euchlanis, Keratella, Kellicottia, Lecane, Polyarthra,* and *Synchaeta*), Acarina (Pionidae), Cladocera (Daphnidae, Bosminidae, and Chydoridae), Calanoida (*Leptodiaptomus, Skistodiaptomus, Limnocalanus, Epischura,* and *Eurytemora*), Cyclopoida (*Diacyclops, Mesocyclops, Tropocyclops,* and *Cyclops*), and Harpacticoida (*Bryocampus*) to be capable of survival in freshwaters. Other taxa (Polychaeta, Gastropoda, Bivalvia, Cirripedia, Euphausiacea, Isopoda, Bryozoa, Echinodermata, and selected Cladocera (Polyphemidae), Calanoida (*Centropages, Temora, Calanus, Acartia,* and *Euchaeta*), Cyclopoida (*Oithona*), Harpacticoida (*Microsetella* and *Parathalestris*), and Amphipoda (*Parathemisto,* and *Gammarus tigrinus*)) we judged to be restricted to salt or brackish water. A third category contained organisms that, because of the taxonomic level to which they were identified, or because of insufficient information on environmental physiology, we were unable to classify according to salinity tolerances; these organisms were not used to determine the effectiveness of midocean exchange. Some illoricate rotifers that could

TABLE 2. Ballast water carried into areas protected by the GLBWCG by 34 vessels that were not in compliance with the GLBWCG (asterisks denote vessels that did not enter the Seaway). Mean tonnage of ballast water was 3667.7 tonnes ($n = 32$).

Date	Noncompliance ballast water carried	Tonnage (tonnes)
91 4 3*	Le Havre (France), Hamburg (Germany); partial exchange in Atlantic	500
91 4 4	Exchanged in coastal Atlantic	2470
91 4 5*	Porta Del Garda (Azores); no exchange	2352
91 4 11	Exchanged in English Channel (<100 m depth)	147
91 4 13	Baltic Sea Ports; partial exchange in Atlantic	1450
91 4 14	Mississippi R. (USA); partial exchange in Atlantic	4626
91 4 16	Limassol (Cyprus); partial exchange in Atlantic	7350
91 4 17	Caen (France); partial exchange in Atlantic	6256
91 4 27	Germany and Atlantic; partial exchange in St. Lawrence R. at Trois Rivières	175
91 4 28	Alexandria (Egypt); partial exchange in Atlantic	6600
91 5 1	Novorossiysk (USSR); partial exchange in Atlantic	6740
91 5 6	Syros (Greece); partial exchange in Atlantic	8200
91 5 7	Riga (USSR); partial exchange in Atlantic	7814
91 5 8	Ghent (Belgium); exchanged in Laurentian Channel east of 61°W	2719
91 5 16	Newark (USA); exchanged in Laurentian Channel west of 63°W	4708
91 5 16	Ravenna (Italy); flushed in Mediterranean Sea	9190
91 5 17	Brooklyn (USA); exchanged in Laurentian Channel east of 61°W	7500
91 5 21	Scotland, Belgium, Spain; partial exchange in Atlantic	1900
91 5 22	Antwerp, North Sea; partial exchange in Atlantic	1010
90 6 13	Montoir (France); partial exchange in Atlantic	6400
90 6 24	Holland, France; exchanged in Laurentian Channel west of 64°W	Unknown
90 7 6	Coastal Atlantic; no exchange	1500
90 7 9	Exchanged in coastal Atlantic	1357
90 8 14	Rotterdam (Netherlands), Mediterranean; partial exchange in Atlantic	1500
90 8 27	Pasajes (Spain); flushed in Atlantic	4300
90 9 13	Il'Ichevsk (USSR); partial exchange in Atlantic	200
90 9 18	Newark (USA); partial exchange and flushed in Atlantic	10946
90 9 25	Hudson R. (USA); no exchange	370
90 10 24	North Sea; discharged in St. Lawrence R.	115
90 11 4	Charlottetown (Canada); no exchange	Unknown
90 11 21	Acapulco (Mexico); no exchange	26
90 11 22	Europe; exchanged in St. Lawrence R. west of 64°W	1000
90 12 1	Hudson R. (USA); no exchange	1680
90 12 4	Rotterdam (Netherlands); exchanged in St. Lawrence R. near Montreal	6266

not be identified after preservation could have been classified either as taxa of uncertain salinity tolerance or, because Thane–Fenchel (1968) and Hollowday (1949) implied that most rotifer taxa are euryhaline, as freshwater-tolerant taxa. The calculated effectiveness value is affected by which assumption is made. We therefore present two effectiveness values representing upper and lower limits. In calculating the lower limit, we used all vessels originating from fresh- or brackish-water ports that reported ballast water exchange (even if exchange was not complete) and assumed all rotifers to be freshwater tolerant. The upper limit was determined using the subset of vessels from fresh- or brackish-water ports that achieved salinities ≥30‰ following exchange, and assuming that unidentified rotifers had unknown salinity tolerance (i.e., omitting them from the analysis).

Results

Rates of Compliance with the GLBWCG

In total, 546 foreign ocean-going vessels entered regions protected by the GLBWCG from May 29, 1990, to May 28, 1991. Ballast water status was not determined for 21 vessels, which were omitted from subsequent calculations. Of the remaining 525 vessels, 236 reported no ballast water, 255 carried ballast water in compliance with the GLBWCG, and 34 carried ballast water not in compliance with the GLBWCG. Including vessels traveling without ballast water in the calculation, 94% ((255 + 236)/525) of vessels were in compliance. Including only vessels carrying ballast water, 88% of vessels were in compliance (255/(525–236)).

Most foreign ocean-going vessels originated from European ports, and ballast water exchange typically occurred in the Atlantic Ocean (60% of vessels) and/or St. Lawrence system (36% of vessels; Table 1). Complete tabular data showing ballast water histories of vessels are presented in Locke et al. (1991) and data deposited with the Canada Institute for Scientific and Technical Information (CISTI).[2]

We estimated that of the 454 foreign ocean-going vessels that entered the Seaway during the study period (102 ships did not travel upriver beyond Montreal), 231 vessels travelling in full or partial ballast each carried, on average, 3115 tonnes of ballast water. Of these vessels, 32 carried ballast water that was not in compliance with the GLBWCG. An additional 223 vessels reported that no ballast water was carried, but these probably

[2]Available, for a nominal fee, from the Depository of Unpublished Data, CISTI, Building M-55, Montreal Rd., Ottawa, ON K1A 0R6, Canada.

TABLE 3. List of zooplankton taxa collected from ballast water, not previously recorded in Locke et al. (1991) (asterisks denote living specimens that were found in one or more ballast tanks).

Rotifera
Kellicottia longispina (Kellicott)*

Polychaeta (Spionidae)
Polydora sp. metatrochophores
Spionida metatrochophores*

Oligochaeta
Chaetogaster diastrophus (Gruithuisen) fragments

Acarina
Mesostigmata
Oribatida
Porohalicaridae (*Stygohalacarus* sp.)

Cladocera
Bosmina ?hagmani (Stingelin)*
Daphnia parvula Fordyce*
Daphnia rosea Sars emend. Richard*

Calanoida
Acartia discaudata (Giesbrecht)*
Acartia longiremus (Lilljeborg)*
Centropages hamatus Lilljeborg*
Clausocalanus lividus Frost and Fleminger*
Eurytemora sp.*
Leptodiaptomus siciloides Lilljeborg*
Metridia sp.*
Paracalanus sp.*
Pleuromamma abdominalis (Lubbock)*
Pleuromamma borealis (Dahl)*
Pleuromamma gracilis (Claus)*
Pseudocalanus acuspes Giesbrecht*
Pseudocalanus elongatus (Boeck)*
Pseudocalanus sp.

Cyclopoida
Acanthocyclops americanus (Marsh)*
Acanthocyclops robustus (Sars)*
Cyclops strenuus (Fischer)*

Harpacticoida
Ectinosomatidae*
Tisbe sp.*

Poecilostomatoida
Oncaea venusta Philippi*
Sapphirina ovatolanceolata Dana*

Amphipoda
Parathemisto gaudichaudi (Guerin)*

carried residual ballast water averaging 158 tonnes. In total, at least 754 799 tonnes of ballast water was carried into the Seaway, of which approximately 14% was not in compliance with the GLBWCG. Anecdotal evidence suggests that much of this ballast water was destined for release into the Great Lakes (D.M. Reid and H.C. van Leeuwen, personal observation).

Lake-going vessels (lakers) also carry ballast water into the Seaway. In 1990, 915 lakers entered the Seaway (St. Lawrence Seaway Authority records). Their cargo and ballast water capacities are similar to those of ocean-going vessels transitting the Seaway (St. Lawrence Seaway Authority records), since maximum size is limited by the dimensions of the Seaway. Ocean-going vessels and lakers, combined, probably carried at least 2.5×10^6 tonnes of ballast water into the Seaway during the study period. Assuming that half the ballast water carried by lakers originated from freshwater portions of the St. Lawrence River, ocean-going vessels and lakers carried approximately 1 million tonnes of freshwater from the St. Lawrence River into the Seaway. In other words, we estimate that about 40% of the ballast water entering the Seaway originated from the St. Lawrence River.

Noncompliance Ballast Water Entering the Great Lakes Drainage

Noncompliance ballast water entered areas protected by the GLBWCG aboard 34 vessels (Table 2). Seven of these vessels had not exchanged their ballast, 17 had completed partial exchanges, and 10 had exchanged their ballast water but not in the areas specified by the GLBWCG. Only one vessel entered the Seaway carrying unexchanged freshwater ballast (from the Mississippi River near New Orleans) and seven others carried partially exchanged freshwater ballast from various sources outside the Great Lakes drainage (Table 2). We conservatively estimate that at least 26 vessels (26/525, or 5% of the total) entering protected areas would have carried unexchanged freshwater ballast prior to the GLBWCG.

Noncompliance ballast water was discharged into the St. Lawrence River and Laurentian Channel outside the alternative ballast exchange area (although not in areas protected by the GLBWCG) by 13 vessels. This ballast water originated from Europe (seven vessels), the United States (four vessels), Australia (one vessel), and the North Sea (one vessel). Some of the discharges probably occurred in areas where lakers or other incoming ocean-going vessels were taking on ballast water. An additional 24 ships discharged or exchanged ballast water in the alternative ballast exchange area in the Laurentian Channel.

Zooplankton in Ballast Water: Effectiveness of Open-ocean Exchange

All ballast tanks sampled contained zooplankton. We collected living representatives of 11 invertebrate phyla (Cnidaria, Platyhelminthes, Rotifera, Nematoda, Annelida, Mollusca, Arthropoda, Tardigrada, Bryozoa, Echinodermata, and Chaetognatha; Table 3; Locke et al. 1991). Although many zooplankton were dead when collected at Montreal, 89% of the ballast tanks sampled contained at least some living zooplankton. On average, 7.5 d had elapsed since water was last added to the tanks, but one tank containing living zooplankton had been in transit for 32 d.

In total, 57 species and at least 50 other taxa of invertebrates were represented in the zooplankton samples. Samples were dominated by copepods, cladocerans, and rotifers, both in numbers of taxa represented and by abundance (Locke et al. 1991; data deposited with CISTI). Maximum abundances per cubic metre (total of living and dead individuals) were 52 748 for *Keratella* sp. (Rotifera), 34 184 for *Bosmina longirostris* (Cladocera), 21 000 for copepod nauplii, and 14 305 for calanoid copepodites. In most cases, however, zooplankton were considerably less abundant than these maxima indicate. Estimates of total zooplankton abundance (all living and dead individuals) in ballast tanks ranged from 21 to >70 000·m^{-3}.

In calculating effectiveness of ballast water exchange, we examined the living zooplankton carried by 24 vessels, originating in fresh or brackish ports, that reported saltwater ballast exchange. If all rotifiers are considered to tolerate freshwater, eight of these vessels carried living zooplankton (rotifiers and cyclopoid and calanoid copepods) that might have survived release in the Great Lakes (Table 4). From this, we calculate that

TABLE 4. Summary of zooplankton, classified by salinity tolerances, collected in ballast tanks containing unexchanged ballast water or ballast water that originated from a fresh- or brackish-water port. Salinity tolerance: F = freshwater tolerant, S = restricted to salt water. Organisms with undetermined salinity tolerance are not shown. Days in tank denotes maximum days-in-transit of ballast water containing living organisms. Ballast exchange category: 1 = ballasted in the St. Lawrence/Great Lakes system ($n = 5$), 2 = originated in freshwater, no exchange ($n = 1$), 3 = originated in brackish water, no exchange ($n = 1$), 4 = originated in coastal salt water, no exchange ($n = 6$), 5 = originated in freshwater, exchanged in fresh or brackish water ($n = 2$), 6 = originated in freshwater, exchanged in salt water ($n = 19$), 7 = originated in brackish water, exchanged in salt water ($n = 5$).

Taxon	Salinity tolerance	Days in tank	No of ships containing living (dead) organisms Ballast exchange category						
			1	2	3	4	5	6	7
Rotifera	F	8	3 (2)	1 (0)	0 (1)	0 (4)	0 (2)	3 (8)	2 (0)
Oligochaeta	F	<7	1 (1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Acarina	F	1–2	1 (1)	0 (0)	0 (0)	0 (0)	0 (1)	0 (1)	0 (0)
Cladocera	F	<7	3 (1)	1 (0)	0 (1)	0 (3)	0 (1)	0 (8)	0 (1)
	S	—	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (1)	0 (0)
Calanoida	F	7–9	5 (0)	1 (0)	0 (0)	0 (1)	0 (0)	3 (0)	1 (0)
	S	14	0 (0)	0 (0)	1 (0)	0 (0)	0 (0)	7 (1)	2 (0)
Cyclopoida	F	<7	4 (0)	1 (0)	0 (0)	0 (0)	0 (0)	1 (2)	0 (0)
	S	8	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	8 (1)	2 (0)
Harpacticoida	F	1	1 (2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	S	10	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	4 (3)	0 (0)
Polychaeta	S	17	0 (0)	0 (0)	0 (0)	2 (0)	0 (0)	1 (2)	2 (0)
Gastropoda	S	8	0 (0)	0 (0)	1 (0)	1 (0)	0 (0)	4 (3)	1 (0)
Bivalvia	S	8	0 (1)	0 (0)	0 (0)	1 (0)	0 (0)	6 (3)	2 (0)
Cirripedia	S	7–9	0 (0)	0 (0)	0 (0)	0 (2)	0 (0)	3 (1)	3 (0)
Isopoda	S	—	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (1)	0 (0)
Amphipoda	S	—	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (1)	0 (0)
Euphausiacea	S	8	0 (0)	0 (0)	0 (0)	0 (1)	0 (0)	1 (0)	0 (0)
Bryozoa	S	8	0 (0)	0 (0)	0 (0)	1 (0)	0 (0)	1 (0)	0 (0)
Echinodermata	S	8	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (1)	0 (0)

ballast water exchange was 67% effective in eliminating all living freshwater-tolerant individuals (from 16 of 24 vessels). However, only 14 of the 24 vessels completed exchanges with final salinities ≥30‰. Considering unidentifiable rotifers to be of undetermined salinity tolerance, two of the 14 vessels contained living freshwater-tolerant taxa (*Eurytemora affinis* and *Asplanchna* sp); thus, effectiveness was 86% (12 of 14 vessels). The range of effectiveness calculated by these two methodologies is therefore 67–86%. Whether one accepts the upper or lower limit, the effectiveness of ballast water exchange is less than 100%.

Living freshwater-tolerant zooplankton of taxa not already present in the Great Lakes were collected only rarely, but four taxa were identified in vessels originating from Europe or the southern United States. *Daphnia rosea, Acanthocyclops robustus,* and *Bosmina hagmani* (the latter species identification is tentative: C. Tudorancea, Department of Biology, University of Waterloo, Waterloo, Ont., personal communication) carried into the Seaway by a ship originating from New Orleans and *Acanthocyclops americanus* carried in ballast water originating from Antwerp, Belgium, have not been recorded in the Great Lakes (Balcer et al. 1984) and must therefore be regarded as potential invaders.

Most of the living freshwater-tolerant zooplankton carried by upbound vessels are already found in the Great Lakes (*Keratella* sp., *Asplanchna* sp., *Synchaeta* sp., *Daphnia retrocurva, Daphnia parvula, Bosmina longirostris, Diaptomus silicoides,* and *Eurytemora affinis* were recorded in the Great Lakes and adjacent waters by Chengalath et al. (1971) and Balcer et al. (1984)).

Discussion

Voluntary ballast water exchange resulted in reasonably good control of dispersal of freshwater organisms but did not eliminate all risk of species invasions to the Great Lakes. In 1990–91, 12% of vessels carrying ballast water did not comply with the GLBWCG. Ballast water exchange in those vessels that did comply with the GLBWCG was not 100% effective, with organisms remaining in the ballast tank after partial, and in some cases even after complete, exchange. Despite these problems and others that we outline below, ballast water exchange is considered by us and others (Williams et al. 1985; Jones 1991) to be a practical quarantine measure.

We found ballast water exchange to be somewhat less satisfactory than we indicated in 1991 (Locke et al. 1991). Our conservative estimate of effectiveness did not change when the sample size of ships from fresh- and brackish-water ports was doubled from 12 (Locke et al. 1991) to 24 (this paper), although we now present a range of values to allow for alternative interpretations of what constitutes a reasonable test of ballast water exchange. However, increases in sampling effort and improved taxonomic resolution have led us to reverse our former conclusion (Locke et al. 1991) that no "new" foreign freshwater zooplankton were present in ballast water entering the Great Lakes. Instead, four species (two cladocerans and two cyclopoid copepods) that were present in the ballast water samples had apparently not been previously recorded in the Great Lakes. Unfortunately, poor documentation of past plankton communities makes it difficult to determine whether a species is new to the region or has simply been overlooked in previous

investigations. Jones (1991) mentioned a similar situation in Australia, and this is the case even in the Baltic Sea, described by Leppäkoski (1984) as "one of the most investigated sea areas in the world".

The effectiveness of ballast water exchange in our present study also appears to be somewhat less than that observed by Williams et al. (1988) for ships travelling from Japan to Australia. This is unexpected given that the exchanges they studied would have succeeded only through physical removal of zooplankton, with little or no physiological stress associated with changing salinities. In their comparison of six pairs of ships, midocean exchange of ballast water eliminated endemic Japanese species that were commonly observed in unexchanged ballast water and reduced the overall numbers of copepod species. In contrast, we found living rotifiers, calanoid copepods, and cyclopoid copepods, potentially capable of surviving in freshwater, in ballast tanks following saltwater exchange (Table 4), although most commonly in tanks where only partial exchange was achieved. Despite the continued presence of these organisms, ballast water exchange probably reduced the numbers of living freshwater-tolerant zooplankton in the ballast tank. Few freshwater species can survive salinities above 10‰ and few marine species are found in salinities below 5‰ (Levinton 1980). There are, however, a few organisms with wide salinity tolerances (e.g., *Eurytemora affinis*, which we have observed living in ballast water of salinities ranging from 6 to 33‰) that might survive the salinity changes associated with a complete midocean ballast water exchange.

Physical limitations of the effectiveness of ballast water exchange include the possible resuspension in ballast water of organisms carried in "residual" water or tank bottom sediments. Residual ballast water (the water that remains at the bottom of a ballast tank after it is "emptied," which cannot be removed because of the position of the pump intake) is of little concern to mariners because the quantities concerned are regarded as insignificant relative to a fully ballasted ship. From a biological point of view, residual water could potentially contain a large number of planktonic organisms. As an extreme case, a ship carrying 468 tonnes (the maximum residual water reported to us) with 70 000 zooplankters·m^{-3} (the maximum abundance we observed) would carry in excess of 32 million zooplankters, including both living and dead individuals. Upon resuspension and mixing with ballast water newly pumped into the tank, these organisms become available for discharge. Resting stages or cysts of both plant and animal species, carried in sediments at the bottom of ballast tanks, may also be resuspended in the ballast water. This is of particular concern in Australia, where toxic dinoflagellate cysts have been introduced in ballast tank sediments (Hallegraeff and Bolch 1991).

Releases of organisms in ballast water discharged into the St. Lawrence River and adjacent harbours, which we estimate provide 40% of the ballast water entering the Great Lakes, might provide another mechanism for invasion. During our 1990 field season, at least 13 ships released ballast water in the river and 14 more in Montreal Harbour (which at that time was not protected by the GLBWCG). At least eight of these vessels discharged water that was not in compliance with the GLBWCG. Several samples we collected from Montreal Harbour in 1990–91 contained marine organisms that almost certainly originated from ballast tanks: a polychaete, an ascidean larva, and another chordate (probably an appendicularian). It is unlikely that these typically stenohaline marine organisms could have survived for long in Montreal Harbour and therefore they could not establish viable populations. Their occurrence does, however, serve as a warning that nonnative species were being discharged in the harbour and presumably also in the river as recently as 1991.

It is almost impossible to say whether current success rates in ballast control will eliminate future invasions. The main criteria for success of potential invaders are probably the ability to tolerate a broad range of environmental conditions (including the ability to complete the full life cycle in freshwater), the capacity for rapid reproduction, and possession of effective dispersal mechanisms. Most of the ballast tank fauna we observed that possess these characteristics are already found in the Great Lakes (e.g., *Eurytemora affinis*, first recorded by Engel (1962)). However, no organism carried into the Great Lakes by ship's ballast should be considered to be completely without risk to the Great Lakes ecosystem. Invasion, however, includes a large element of chance. According to Vermeij (1991), only a small proportion of species in any biota are capable of successful invasions; during episodes of marine and terrestrial biotic interchanges (e.g., formation or dissolution of land bridges) that have occurred during the past 20 million years, between 1 and 22–46% (but most often <15%) of any given biota have successfully invaded a newly available habitat. The vagaries of meeting a mate, avoiding predators, and finding food are difficult to quantify into a model of invasion probability.

Our view of ballast water exchange is that it acts as a filter to reduce the numbers or types of organisms dispersed rather than as an impassible barrier to dispersal. The probability of invasions has most likely been reduced by the GLBWCG. These guidelines might have been able to prevent invasions by organisms such as zebra mussels and fishes. Certain other invaders such as *Bythotrephes* or other species with resting eggs that could be transported in sediments might not have been prevented. Development of techniques to further minimize the risk of species invasion to the Great Lakes is advisable.

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Effects of Exercise Conditioning on Stress Responses and Recovery in Cultured and Wild Young-of-the-Year Striped Bass, *Morone saxatilis*

Paciencia S. Young and Joseph J. Cech, Jr.[1]

Wildlife and Fisheries Biology, University of California, Davis, CA 95616, USA

Young, P.S., and J.J. Cech, Jr. 1993. Effects of exercise conditioning on stress responses and recovery in cultured and wild young-of-the-year striped bass, *Morone saxatilis*. Can. J. Fish. Aquat. Sci. 50: 2094–2099.

Exercise conditioning at 1.2–2.4 body lengths·s^{-1} for 60 d significantly improved physiological responses to and decreased recovery time from capture, net confinement, and crowding (collectively, "handling") stress in cultured and wild young-of-the-year striped bass, *Morone saxatilis*. Plasma cortisol increased dramatically 0.5 h after acute handling in all treatments. However, cortisol returned to prestress levels 4 h after handling in exercise-conditioned cultured and wild fish but not in the respective unexercised fish. Handling stress in all groups of fish also resulted in hyperlacticemia. Faster clearance of plasma lactate following handling stress was shown in exercise-conditioned cultured and wild striped bass compared with unexercised fish. Handling stress resulted in a rapid hemoconcentration as indicated by increases in osmolality and hematocrit levels. Osmotic imbalance was less severe in exercise-conditioned cultured and wild fish than in unexercised fish. These improved stress responses and enhanced recovery in young-of-the-year striped bass should result in increased survival of both cultured and wild fish after transport and stocking into the natural environment.

Le conditionnement par exercice, à 1,2–2, 4 bl·s^{-1} pendant 60 jours chez des jeunes de l'année de bar rayé, *Morone saxatilis*, sauvages et d'élevage, a nettement amélioré les réactions physiologiques et réduit le temps de récupération après le stress causé par la capture, le confinement dans le filet et l'entassement (globalement, « stress de manipulation »). Le cortisol du plasma a marqué une hausse spectaculaire 0,5 h après manipulation aiguë dans tous les traitements. Toutefois, il est redescendu au niveau d'avant le stress 4 h après la manipulation chez les poissons sauvages et d'élevage conditionnés par exercice, mais pas chez les poissons non conditionnés. Dans tous les groupes de poissons, le stress de manipulation a aussi causé une hyperlactacidémie. Une élimination plus rapide du lactate du plasma à la suite du stress de manipulation a été observée chez les poissons conditionnés par rapport aux poissons non conditionnés. Le stress de manipulation provoquait une rapide hémoconcentration que révélaient des augmentations de l'osmolalité et de l'hématocrite. Le déséquilibre osmotique était moins grave chez les poissons conditionnés que chez les autres. Ces améliorations dans la réaction au stress et dans la récupération chez les jeunes de l'année de bar rayé devraient permettre d'accroître la survie des poissons sauvages et d'élevage après le transport et l'ensemencement dans le milieu naturel.

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High rates of mortality in fish during harvest and/or stocking into the natural environment have been attributed to stress caused by several factors, among which are capture, net confinement, and crowding (reviewed by Schreck 1981). These activities have been demonstrated to cause acute stress in young-of-the-year (YOY) striped bass, *Morone saxatilis*, resulting in endocrinological changes, hyperlacticemia, and osmotic imbalance. It took at least 4 h in a 1.0% NaCl recovery environment and longer in fresh water for plasma cortisol and lactate to return to prestress levels and for hematocrit and osmolality to be stabilized (Young and Cech 1993a).

The steep decline of the Bay Delta striped bass populations in California has concerned the sportfishing public, the associated industries and organizations, as well as the California Department of Fish and Game (CDFG), Department of Water Resources, and State Water Resource Control Boards. While various factors responsible for the decline were being determined, young striped bass were mitigatively stocked into the Sacramento – San Joaquin Delta system. Between 1981 and 1991, about 11 million striped bass were released (N. Villa, CDFG, personal communication). However, in spite of these mitigative efforts, the striped bass populations continue to decline at an alarming rate. We believe that cultured (hatchery) and wild (salvaged from screens guarding water export pumps and held in a grow-out facility for 2–3 mo) YOY striped bass stocked into the Delta system had poor survival partly because of the stress involved in stocking, although CDFG tried several ways (e.g., addition of salt in the transport environment) to minimize the stress. We hypothesized that exercise conditioning could improve the stress responses and enhance recovery in cultured and wild YOY striped bass, thereby increasing their survival during and after stocking.

Exercise conditioning has been shown to improve growth, swimming performance, and muscular development in YOY striped bass (Young and Cech 1993b). It has also been shown to lower oxygen consumption rates (Woodward and Smith 1985)

[1]Author to whom reprint requests should be addressed.

and increase the aerobic capacity of red and white muscle in rainbow trout, *Oncorhynchus mykiss* (David et al. 1986). Woodward and Smith (1985) found lower levels of epinephrine, norepinephrine, cortisol, and glucose in resting rainbow trout after 3 wk of a swimming exercise program. Lackner et al. (1988) showed a faster return to prestress levels of glycolytic metabolite concentrations in exercise-conditioned chub, *Leuciscus cephalus*, after acute handling. Appropriate stress responses regulate physiological systems and may increase fish survival after transport and stocking into the natural environment (Carmichael et al. 1984; Nikinmaa et al. 1984).

The main objective of this study was to test the hypothesis that exercise conditioning improves stress responses and enhances recovery from stress in cultured and wild YOY striped bass. The study was aimed at producing fish with diminished stress responses (i.e., a stressor would be less severe for a conditioned fish than for an unconditioned one) and with faster recovery from handling stress.

Materials and Methods

Sources of fish used in the study and the method used for exercise conditioning have been described elsewhere (Young and Cech 1993b). Briefly, cultured YOY striped bass were obtained from CDFG's Central Valley Hatchery in Elk Grove, California; wild YOY were salvaged from screening devices guarding water export pumps at Byron, California, and held for a week at the Department of Water Resources grow-out facility in Byron. Fish from each source were individually weighed and randomly distributed among 12 plastic raceways. Each raceway, measuring 150 cm long, 30 cm wide, and 25 cm deep, had a screen placed cross-sectionally 30 cm from each end; the inner compartment was divided longitudinally by a PVC sheet into two compartments (90 × 15 cm each). Fifteen cultured fish were stocked in one compartment and 15 wild fish in the other compartment. Fish in six raceways were exercise conditioned (after 1 d of acclimation) and the rest served as controls. Exercise conditioning was achieved by using submersible pumps. A Teel submersible pump with an attached flow diffuser was placed beyond the screen at each end of each of the six raceways to generate and maintain the desired water velocity. At the start of exercise conditioning, the water velocity was 6–12 $cm \cdot s^{-1}$ (equivalent to 1.2–2.4 body lengths $(bl) \cdot s^{-1}$). Within a compartment, the water velocity was about 12 $cm \cdot s^{-1}$ nearest the pump and about 6 $cm \cdot s^{-1}$ farthest from the pump. Water depth was 15 cm with a continuous flow of air-equilibrated well water at 2 $L \cdot min^{-1}$. Aeration was provided at each end of the raceway. Water velocities were determined using a Marsh McBirney flowmeter and no "dead" spaces were found within the rearing compartments. The rest of the fish from each source were reared in identical raceways (without pumps) with a water velocity <1 $cm \cdot s^{-1}$.

After 30 d, the water velocity in exercise-conditioned fish was increased to 10–19 $cm \cdot s^{-1}$ by moving the pumps nearer the fish compartments. The increase in flow was necessary to correspond to the increase in fish size so that flow was equivalent to 1.2–2.4 $bl \cdot s^{-1}$.

At the start of conditioning, pumps were turned on only between 08:00 and 17:00 for 5 d. Throughout the rest of the conditioning, they were left on (except during feeding (20 min) and cleaning (20 min)) all the time. Fish were fed with Silver Cup No. 2 trout pellets at about 5% body weight four times daily for the first 2 wk, three times daily for the next 2 wk, and twice daily for the rest of the experimental period. Fecal matter and extra food were removed before pumps were turned back on. None of the fish died during the 60-d conditioning period.

Fish were kept in a 12 h light : 12 h dark photoperiod regime. Temperature, dissolved oxygen content, and ammonia content of the water were monitored to ensure high-water quality. Temperature (YSI telethermometers, max:min thermometers) in the raceways fluctuated from a mean minimum of 18.3°C (±0.07) at night to a mean maximum of 24.9°C (±0.06) during the day with a daily mean temperature of 21.7°C (±0.57). Dissolved oxygen content (Nester DO meter) remained high at 7.6 $mg \cdot L^{-1}$ (±0.03) and ammonia content (salicylate–hypochlorite spectrophotometric method, Bower and Holm–Hansen 1980) was always low at 0.13 ppm (±0.01) throughout the 60-d conditioning period. None of these variables differed significantly among treatments.

At the end of the conditioning period, two fish from each raceway were individually sampled as "resting" samples. Two groups of fish (five fish per group) per raceway were then separately net-confined in a 20 × 15 × 18 cm dip net over a shallow pan (18 × 26 cm) containing 4-cm-deep aerated (airstone directly beneath net) water for 90 s, hereafter referred to as "handling." Individual fish from the net were then immediately distributed into individual 28-cm-diameter containers with 10 L of nonflowing aerated 1.0% NaCl solution (Young and Cech 1993a). This procedure was used because during stocking, YOY striped bass are loaded to transport trucks containing nonflowing aerated water with salt added. Blood samples were taken by caudal transection (Houston 1990) immediately after net confinement (0 h) and during the postconfinement recovery period at 0.5, 2, 4, and 24 h. Hematocrit was read and plasma from each fish was stored in freezer vials.

Plasma was later thawed for cortisol, lactate, and osmolality measurements. Cortisol levels were measured using a modified enzyme immunoassay (ELISA, Munro and Stabenfeldt 1985). The protein fraction of 25 µL of plasma was precipitated by the addition of 500 µL of absolute ethanol. After vortexing and centrifugation, 50-µL aliquots of the ethanol fraction were dried under air. Before assay, 250 µL of cortisol – horseradish peroxidase conjugate was added and 50-µL aliquots of each sample were taken in duplicate for the assay. This assay sensitivity was 0.25 $pg \cdot well^{-1}$. Interassay variation ranged from 7 to 13%, and intraassay variation ranged from 5 to 10%. It should be noted that cortisol antibody cross-reacts with prednisolone (9.9%), prednisone (6.3%), compound S (6.2%), and cortisone (5.0%) and with other fish steroids (at less than 1%). Lactate levels were determined using a calibrated YSI model 27 lactate analyzer. Osmolality was measured using a calibrated Wescor 5100B vapor pressure osmometer.

Analyses of variance (ANOVA) and Tukey's test using SYSTAT software tested for significant differences among treatments and sample times. Cortisol values were transformed to $\log_{10}$ before conducting ANOVA and Tukey's test to increase homogeneity of variances. The value of 1 was added to each cortisol datum before log transformation to avoid negative values.

All n for resting and for hematocrit data were 12; for plasma cortisol, lactate, and osmolality immediately after handling stress, and after 0.5 and 2 h, n varied from 9 to 12 due to inadequate plasma volume in some samples.

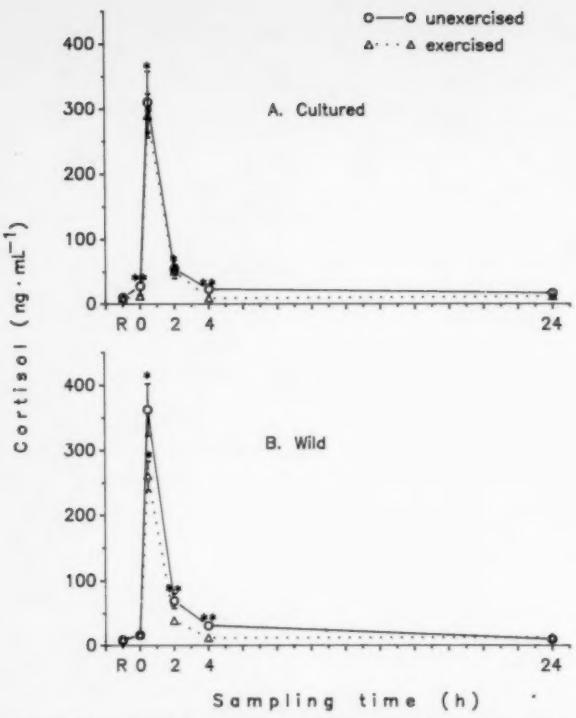

FIG. 1. Mean (±SEM) plasma cortisol of unexercised and exercised (A) cultured and (B) wild fish at resting (R), immediately after net confinement (0), and 0.5, 2, 4, and 24 h after net confinement (bars = SEM; *values for unexercised and exercised fish significantly greater than resting values, $P < 0.03$; **significantly greater than those of exercised fish, $P < 0.05$).

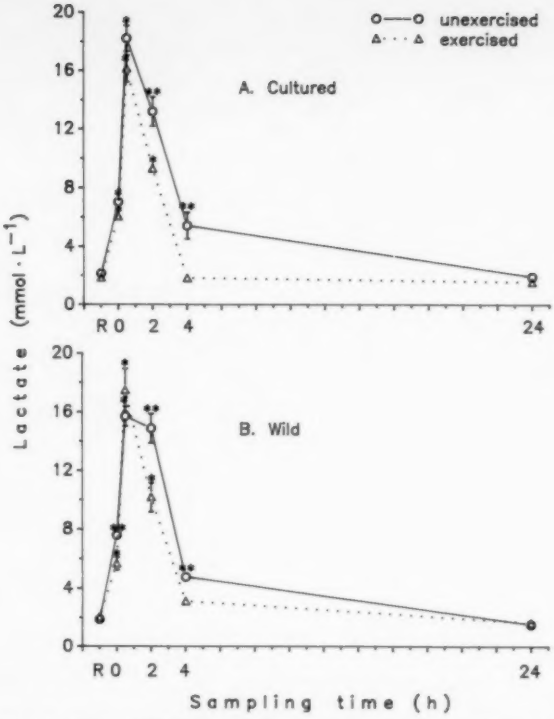

FIG. 2. Mean (±SEM) plasma lactate of unexercised and exercised (A) cultured and (B) wild fish at resting (R), immediately after net confinement (0), and 0.5, 2, 4, and 24 h after net confinement (bars = SEM; *values for unexercised and exercised fish significantly greater than resting values, $P < 0.001$; **significantly greater than those of exercised fish, $P < 0.04$).

Results

Cortisol Responses

Capture, net confinement, and crowding in a shallow pan of water for 90 s resulted in a dramatic increase in plasma cortisol in all fish followed by recovery at different rates. Resting (prestress) cortisol levels did not vary among treatment groups. Immediately after handling, there was a slight but significant ($P < 0.05$) increase in plasma cortisol levels in unexercised cultured YOY striped bass but not in the exercise-conditioned fish (Fig. 1A) or in the unexercised and exercise-conditioned wild fish (Fig. 1B). After 0.5 h, cortisol levels increased 32-fold in both unexercised and exercise-conditioned cultured YOY striped bass, 42-fold in unexercised wild fish, and 26-fold in exercise-conditioned wild fish. After 2 h, cortisol levels in unexercised and exercise-conditioned cultured and wild fish were still significantly ($P < 0.05$) higher than prestress levels, and levels in exercise-conditioned wild fish were significantly ($P < 0.04$) lower than those in unexercised wild fish. After 4 h, cortisol levels in exercise-conditioned cultured and wild fish had returned to prestress levels, while those of the unexercised fish were still significantly ($P < 0.05$) higher than prestress levels. Furthermore, 4-h levels of cortisol in exercise-conditioned cultured and wild fish were also significantly ($P < 0.05$) lower than those in the corresponding unexercised fish. After 24 h, cortisol levels in all fish returned to prestress levels.

Lactate Responses

Mean plasma lactate increased to peak levels 0.5 h after handling stress and decreased at different rates during recovery. Resting lactate levels did not vary among treatment groups (Fig. 2). Lactate levels in unexercised and exercise-conditioned cultured and wild fish rose about threefold immediately after handling stress. After 0.5 h, plasma lactate in unexercised and exercise-conditioned cultured and wild fish continued to increase eightfold. At 2 h, lactate levels in unexercised and exercise-conditioned cultured and wild fish were still significantly ($P < 0.001$) higher than prestress levels. Moreover, lactate levels of the unexercised cultured and wild fish were significantly ($P < 0.002$) higher than those of the corresponding exercise-conditioned fish. At 4 h, lactate levels in exercise-conditioned cultured and wild fish had returned to prestress levels, while those in unexercised fish were significantly ($P < 0.03$) higher than prestress levels and significantly ($P < 0.04$) higher than the 4-h exercise-conditioned values. All fish lactate values were at prestress levels after 24 h.

Osmolality Responses

Plasma osmolality in cultured and wild fish showed similar responses to handling stress and recovery (Fig. 3). Osmolality did not vary among treatment groups of resting fish. Levels in unexercised and exercise-conditioned cultured and wild fish rose

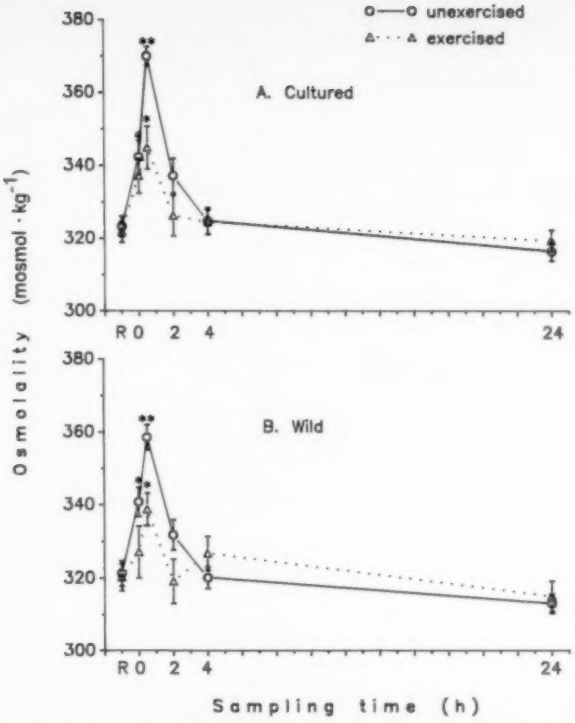

FIG. 3. Mean (±SEM) plasma osmolality of unexercised and exercised (A) cultured and (B) wild fish at resting (R), immediately after net confinement (0), and 0.5, 2, 4, and 24 h after net confinement (bars = SEM; *significantly greater than resting values, $P < 0.01$; **significantly greater than those of exercised fish, $P < 0.008$).

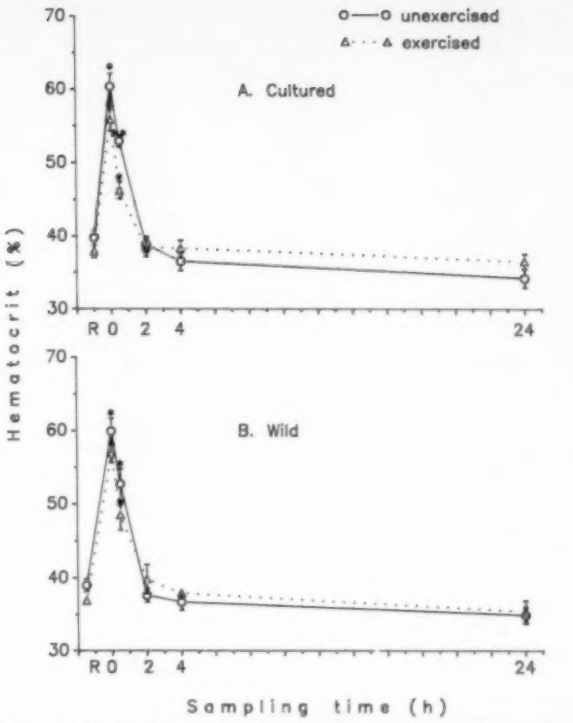

FIG. 4. Mean (±SEM) hematocrit of unexercised and exercised (A) cultured and (B) wild fish at resting (R), immediately after net confinement (0), and 0.5, 2, 4, and 24 h after net confinement (bars = SEM; *values for unexercised and exercised fish significantly greater than resting values, $P < 0.001$; **significantly greater than those of exercised fish, $P < 0.002$).

immediately after handling stress and continued to increase to a peak at 0.5 h, where levels in unexercised fish (both cultured and wild) were significantly ($P < 0.03$) higher than those of exercise-conditioned fish. At 2 h, all osmolalities were not significantly different from prestress levels, although those in unexercised fish still tended to be higher ($P < 0.07$) than prestress levels and those of the exercise-conditioned fish. At 4 and 24 h, all osmolalities were not significantly different from prestress levels.

Hematocrit Responses

Hematocrit levels in unexercised and exercise-conditioned cultured and wild fish rose 1.5-fold immediately after handling stress and decreased during recovery at different rates (Fig. 4). At 0.5 h after handling stress, all hematocrit levels decreased from 0 h levels but were still significantly ($P < 0.001$) higher than prestress levels. Hematocrits in exercise-conditioned cultured fish (but not in wild fish) at 0.5 h were significantly ($P < 0.002$) lower than those in the unexercised fish. At 2 h, all hematocrits had returned to prestress levels and remained there through 24 h after handling stress.

Discussion

Exercise conditioning at 1.2–2.4 bl·s^{-1} for 60 d significantly improved physiological responses to and recovery from handling stress for both cultured and wild YOY striped bass. Plasma cortisol of YOY striped bass in all treatments increased dramatically 0.5 h after handling stress. However, cortisol levels 4 h after handling stress returned to prestress levels in exercise-conditioned cultured and wild fish but not in unexercised fish. This indicates that exercise conditioning in YOY striped bass enhanced recovery from stress in terms of a faster return of plasma cortisol to prestress levels. It has been demonstrated that prolonged elevation of plasma cortisol is a major factor contributing to the damaging effects of stress on survival (Pickering 1992). Elevated plasma cortisol indicates reduced relative fitness of the stressed fish and results in lower survival rates to adulthood for fish stocked while their circulating cortisol titers are still high (Schreck et al. 1989). If cortisol levels in stressed fish are more quickly reduced to resting levels, the effects of stress could consequently be minimized and survival improved.

The apparent lack of differences in the stress responses between cultured and wild fish is probably due to the fact that broodstock used for hatchery production of striped bass is taken from the wild so that there is a lack of genetic "domestication." In rainbow trout, stress-induced changes in plasma cortisol were more extreme in wild fish than in cultured fish (Woodward and Strange 1987). This difference was attributed to years of artificial selection of rainbow trout through generations of culture.

Handling stress in unexercised and exercise-conditioned cultured and wild YOY striped bass resulted in hyperlacticemia.

Faster clearance of plasma lactate following handling stress was observed in exercise-conditioned cultured and wild striped bass compared with unexercised fish. Faster clearance of lactate in exercise-conditioned fish following stress was also observed in young Atlantic salmon, *Salmo salar* (Wendt and Saunders 1973), rainbow trout (Hammond and Hickman 1966; Woodward and Smith 1985; Pearson et al. 1990), and chub (Lackner et al. 1988). Such higher lactate clearance rates coupled with greater aerobic capacity (Farlinger and Beamish 1978; Nahhas et al. 1982; Davie et al. 1986; Totland et al. 1987) in exercise-conditioned fish could be responsible for better swimming performance and stamina (Davison 1989). Indeed, our previous study on YOY striped bass (Young and Cech 1993b) showed a significantly increased mean critical swimming velocity in exercised cultured and wild fish compared with unexercised fish. Better swimming performance improves feeding efficiency and escape response from predators, as well as the capacity to maintain station against a current (reviewed by Beamish 1978). These factors are important for the survival of fish in the natural environment.

The faster clearance rates of plasma cortisol and lactate in exercise-conditioned striped bass was probably due to increased perfusion rates in the muscles. Higher capillary density in red and white muscle fibres has been shown in exercise-conditioned rainbow trout (Davie et al. 1986) as has increased perfusion in working muscles in exercising fish to meet metabolic requirements (Wood and Perry 1985). We speculate that a higher capillary density in red and white muscle fibres of exercise-conditioned YOY striped bass facilitates increased perfusion and enhances clearance of plasma cortisol and lactate.

Handling stress resulted in rapid hemoconcentration as indicated by increases in osmolality and hematocrit levels in YOY striped bass. These same observations were reported in a previous study (Young and Cech 1993a). Our results showed that increases in osmolality in exercise-conditioned cultured and wild fish were not as high as levels in unexercised individuals, indicating improved osmoregulation associated with exercise conditioning. No hemodilution was indicated during the 24-h recovery period. This is probably due to the recovery environment being a 1.0% NaCl solution.

Past studies on other species indicate that the rapid hemoconcentration we observed in YOY striped bass immediately after handling may be due to one or more of three factors. The first involves fluid shifts within the fish, wherein plasma water moves into muscles and erythrocytes caused by increased production of catecholamines (Nikinmaa et al. 1984; Wood and Perry 1985). Reid and Perry (1991) have recently shown that increased cortisol enhances catecholamine-erythrocytic binding by activating catecholamine receptors on the erythrocytic membrane in rainbow trout. The second factor is water loss due to increased diuresis (Wood and Randall 1973). The third factor is splenic contraction (Yamamoto 1989; Wells and Weber 1990). Our results showed dramatic increases in hematocrit levels in both unexercised and exercise-conditioned cultured and wild fish; however, increases in osmolality levels in exercised cultured and wild fish were not as high as in the unexercised fish. This suggests that the increased hematocrit levels in exercised cultured and wild fish were due more to splenic contraction than to fluid shifts or water losses. Farlinger and Beamish (1978) reported decreased blood volume in unexercised largemouth bass, *Micropterus salmoides*, following periods of swimming at critical speeds, while exercise-conditioned fish exhibited a relatively stable blood volume. Decreases in blood volume in our unexercised striped bass could have resulted from fluid shifts, indicating better osmoregulation in the exercise-conditioned fish.

Capture, net confinement, and crowding in YOY striped bass constitute a stressor that results in elevations of plasma cortisol (primary stress response) and plasma lactate, osmolality, and hematocrit (secondary stress responses). These adaptive responses to stress require energy to restore homeostasis or to offset the effects of the stressor (Beitinger and McCauley 1990). We have demonstrated that exercise conditioning in YOY striped bass resulted in improved stress responses and faster recovery, i.e., better osmoregulatory functions and a faster return to resting levels in plasma cortisol and lactate. These improved stress responses and faster recovery should result in better energy conservation and increased survival of fish after transport and stocking into natural environments. Both cultured and wild YOY striped bass benefit from exercise conditioning. This is the first demonstration of exercise-conditioning effects on the stress responses of a percichthyid fish. Exercise conditioning of young striped bass should assist in the survival and migratory return of this anadromous species as has been demonstrated in Atlantic salmon smolts (Wendt and Saunders 1973) and brown trout, *Salmo trutta* (Creswell and Williams 1983).

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First Cohort of Young-of-the-year Dungeness Crab, *Cancer magister*, Reduces Abundance of Subsequent Cohorts in Intertidal Shell Habitat[1]

Miriam Fernandez, David Armstrong, and Oscar Iribarne

School of Fisheries WH-10, University of Washington, Seattle, WA 98195, USA

Fernandez, M., D. Armstrong, and O. Iribarne. 1993. First cohort of young-of-the-year Dungeness crab, *Cancer magister*, reduces abundance of subsequent cohorts in intertidal shell habitat. Can. J. Fish. Aquat. Sci. 2100–2105.

Arrival and settlement of successive cohorts of Dungeness crab, *Cancer magister*, megalopae were observed in Grays Harbor, Washington, in 1991. The first cohort of megalopae entered the estuary between May 15 and 20 and settled in ("occupied") previously constructed artificial, intertidal oyster shell habitats at densities ranging from 155 to 196 first instar juvenile (J1) crabs·m^{-2}. Subsequently, a second set of shell habitats was constructed that did not contain crabs of the first cohort ("unoccupied"). Between June 15 and 18, when crabs of the first cohort were a mix of second and third instars, a second cohort of megalopae settled in both occupied and unoccupied shell habitat at respective J1 crab densities of 9–37 and 168–298 crabs·m^{-2}. The possible roles of cannibalism, competition, and conspecific avoidance are proposed as alternative hypotheses to explain significantly lower density of the second cohort in shell habitats previously occupied by larger conspecifics of the first cohort. Based on laboratory experiments, we suggest that cannibalism is a plausible explanation of our field observations.

L'arrivée et l'installation de cohortes successives de mégalopes de crabe dormeur du Pacifique, *Cancer magister*, ont été observées à Grays Harbor, État de Washington, en 1991. La première cohorte de mégalopes est entrée dans l'estuaire entre le 15 et le 20 mai et s'est installée dans des habitats artificiels déjà construits dans la zone intertidale avec des coquilles d'huître (« habitats occupés ») à des densités allant de 155 à 196 crabes juvéniles de premier stade (J1)·m^{-2}. Par la suite a été construite une deuxième série d'habitats de coquilles qui ne contenaient pas de crabes de la première cohorte (« habitats inoccupés »). Entre le 15 et le 18 juin, alors que les crabes de la première cohorte constituaient un mélange de deuxième et de troisième stades, une deuxième cohorte de mégalopes s'est installée dans les habitats « occupés » et dans les habitats « inoccupés », à des densités respectives de crabes J1 de 9–37 et de 168–298 individus·m^{-2}. Nous avons examiné le rôle possible du cannibalisme, de la concurrence et de l'évitement intraspécifique pour expliquer le fait que la densité était nettement plus faible pour la deuxième cohorte dans les habitats déjà occupés par des congénères de plus grande taille provenant de la première cohorte. À partir d'expériences de laboratoire, nous considérons que le cannibalisme est une explication plausible des observations que nous avons faites sur le terrain.

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Recruitment variability is a central problem in fishery sciences and a major source of uncertainty in fisheries management. Two general approaches have been used to address recruitment problems: the empirical approach and the process-oriented approach (Sissenwine 1984). The empirical approach involves fitting statistical relationships between recruitment and physical and/or biological variables (e.g., Flowers and Saila 1972); the process-oriented approach identifies the causes of egg, larval, or postlarval mortality and the factors that contribute to variability of such prerecruit mortality. Relationships between larval survival and food availability (Lasker 1975), and larval abundance and predation or cannibalism (e.g., Hunter and Kimbrell 1980; Hunter 1981) are the most studied processes.

Recruitment variability of Dungeness crab, *Cancer magister*, has been explained by statistical relationships between landings (as a proxy for planktonic larval abundance years prior) and physical variables. Winnor (1986) found a multiple correlation between Dungeness crab landings in the San Francisco area and a 4-yr lag in January–April sea level, sea surface temperature, and wind. Also, a positive relationship was found between upwelling and Dungeness crab landings in Washington, Oregon, and northern California (Peterson 1973). Currents and their velocities determine patterns of spatial distribution of crab megalopae and their subsequent ability to move onshore (Jamieson et al. 1989). In Washington and Oregon, variability in settlement of 0+ crabs has been linked to the extent of northerly transport during larval development, based on circulation patterns and winds (Jamieson and Armstrong 1991; McConnaughey et al. 1992).

Processes involving predation and cannibalism have been suggested as determinants of Dungeness crab recruitment. Silver (*Oncorhynchus kisutch*) and king (*O. tshawytscha*) salmon prey heavily on Dungeness crab megalopae (Reilly 1983). Prince and Gotshall (1976) found Dungeness crab megalopae and postlarval instars to be the most important food items for copper rockfish, *Sebastes caurinus*, in Humboldt Bay, California. Armstrong (1991) reported high incidence of predation by Pacific staghorn

[1]Contribution 866 from the School of Fisheries, University of Washington, Seattle, WA 98195, USA.

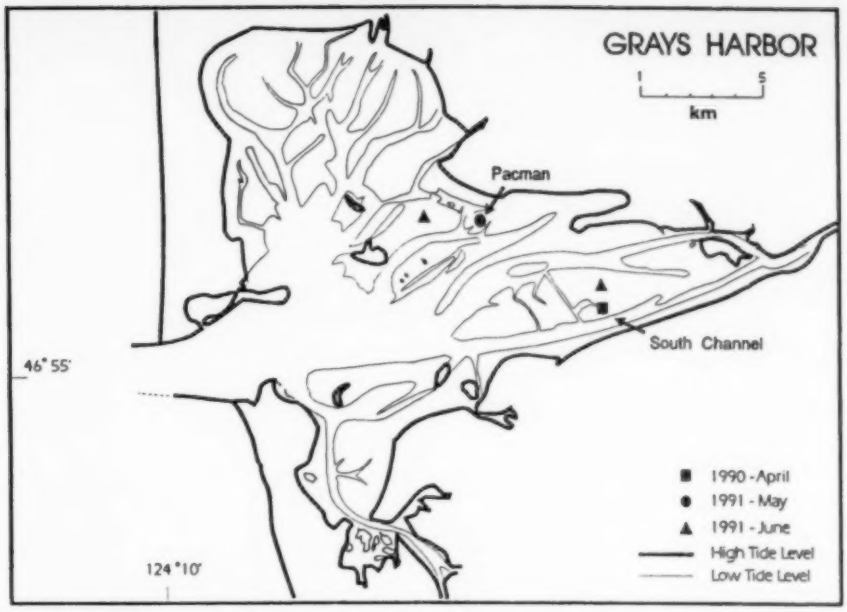

FIG. 1. Grays Harbor Estuary showing the location of the experimental areas South Channel and Pacman. Dates in the lower right indicate shell deployment time.

sculpin, *Leptocottus armatus*, on early Dungeness instars in the Grays Harbor estuary and estimated that, at the population scale, over 50% of a settling crab year-class could be lost through sculpin predation. Cannibalism among young-of-the-year (YOY) individuals under laboratory conditions (Jacoby 1983) and by larger crabs on their smaller conspecifics has been reported (Gotshall 1977; Stevens et al. 1982), and cannibalism by older age groups on juveniles has been suggested as a cause of the cyclic behavior of Northern California Dungeness crab populations (Botsford and Wickham 1978).

In the intertidal area of Grays Harbor, Washington, YOY Dungeness crabs (particularly the first six instars) are found among bivalve shells at higher densities than on adjacent exposed substrata (Dumbauld et al. 1993). Local density of Dungeness crabs has been successfully enhanced by constructing oyster shell habitat in the intertidal zone (Armstrong et al, 1991; Dumbauld et al. 1993). Density of first instar juvenile (J1) crabs in this artificial habitat and in natural, dead shell assemblages (*Mya arenaria*) may exceed 300 crabs·m^{-2} (Armstrong et al. 1991). Such densities suggest the potential for strong intraspecific agonistic interaction, which might be exacerbated by the episodic pattern of settlement by Dungeness crab megalopae; at least two cohorts of megalopae per annum have been detected in Puget Sound (Orensanz and Gallucci 1988; Dinnel et al. 1993), in Grays Harbor (personal observation), and along the outer coast (McConnaughey et al. 1992). In this paper, we show that the first cohort of YOY Dungeness crabs to settle annually in shell habitat may control survival and abundance of subsequent cohorts of settling megalopae by either cannibalism or other yet untested processes of competition or conspecific avoidance.

Material and Methods

Field Observations

Since April 1990, a variety of shell plots have been constructed by the U.S. Army Corps of Engineers (COE) in the intertidal of Grays Harbor to study issues including techniques of handling large quantities of shell, integrity of the habitat over time, and, most importantly, the mean summer density of YOY crabs that settle into this habitat (see McGraw et al. 1988 and Wainwright et al. 1992 for further details). Timing and spacing of shell habitat construction in 1991 provided a unique opportunity to study the interaction between successive settling cohorts on a spatial scale rarely available in experimental projects. Prior to settlement of the 1991 year-class, two sites were selected for detailed sampling of YOY density. These were a 4000-m^2 shell plot at South Channel constructed in April 1990 and a 1600-m^2 shell plot constructed in May 1991 on a tidal flat called "Pacman" (Fig. 1). In all cases, plots of shell habitat were constructed in the intertidal with empty oyster shell (*Crassostrea gigas*) delivered by barge at high tide and spread to a relatively uniform thickness of about 20 cm. The first cohort of megalopae entered the estuary and settled to ("occupied") South Channel (SC-O) and Pacman (PC-O) sites between May 15 and 20 during the full moon. Two smaller plots were constructed in early June, one at Pacman (100 m^2) and one at South Channel (225 m^2), after the first cohort settled, and so were "unoccupied" by the first cohort (PC-U, SC-U) as the second cohort entered the estuary and settled in late June. Tidal elevation differed between experimental sites (South Channel and Pacman) by 0.3 m but was constant across individual shell plots and between occupied and unoccupied shell plots within each of the two sites. Since this study was done within the construction framework of the COE, we could not provide for replication of occupied and unoccupied plots at each

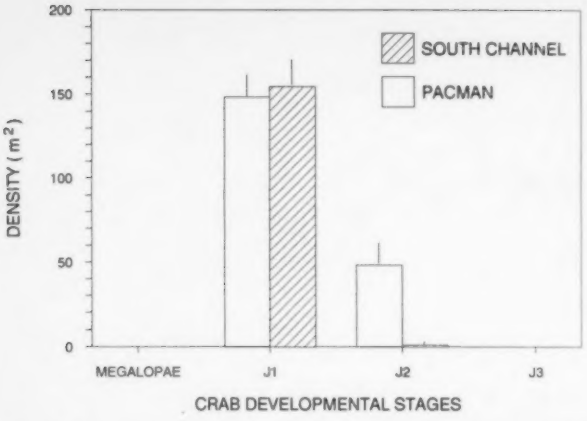

FIG. 2. Mean density of megalopae, J1, J2, and J3 Dungeness crabs in sites SC-O (hatched bars) and PC-O (open bars) shortly after the first peak of settlement (May 28). Bars indicate mean density; lines indicate 1 SE. J1, first instar crab; J2, second instar crab; J3, third instar crab.

site or control their size. However, we know from previous work (Armstrong et al. 1991, 1992) that plot size at the scale of this study does not affect crab density.

To estimate density of megalopae and first (J1) to third instar (J3) crabs, samples were taken at plots SC-O ($N = 9$) and PC-O ($N = 6$) on May 28, at plots PC-O ($N = 10$) and PC-U ($N = 11$) on June 26, and at plots SC-O ($N = 10$) and SC-U ($N = 18$) on June 27. Samples were not taken on the edge of shell plots (1-m-wide stripe) to avoid variability in crab density due to decreased crab survival in those areas (personal observation). Random 0.1-m^2 samples were taken at each plot and date by excavating all shell to the sediment layer plus about 5 cm depth. Shell and mud were sieved through a 2-mm mesh which retained all crab stages. Crabs were counted and carapace width (CW) was measured between the tenth anterolateral spines. The first three instar stages of juvenile Dungeness crabs were identified based on the criteria established by analysis of polymodal frequency distribution of juvenile crabs (Dinnel et al. 1993): J1, 6–8 mm; J2, 10–12.5 mm; and J3, 13–15 mm.

The density of crabs per square metre was calculated for each treatment (occupied and unoccupied) plot by site and date, and the means were used as the data for statistical analysis. A factorial analysis of variance (ANOVA) on the square-root-transformed mean densities, with treatments crossed with sites, was performed to test the null hypothesis of no difference in mean density of crabs of the second cohort between treatments and between sites. A crossed-factorial analysis on the square-root-transformed mean densities was also used to test for differences in mean density of crabs of the first cohort between treatment and between sites. Square root transformation was used to "remove" interaction effect (Winer 1971) between treatment and site. Based on graphical assessment, the interaction effect of the square-root-transformed data between treatment and site was assumed not significant.

Laboratory Experiments

Shell selection

This experiment was designed to test whether megalopae settle preferentially in shells that had been in place longer than a month ("weathered"; with attached fouling) or in new ("clean") shells, since the two categories of shell plot studied in situ were constructed months apart. Ten 40-L glass aquarium were conditioned with a 5-cm layer of mud collected in the intertidal zone and filled with estuarine water (25–27‰ salinity). Half of the bottom of each aquarium was covered with a layer of weathered shells and the other half with a layer of clean oyster shells. Megalopae collected from the estuary by plankton tows were placed in groups of 10 in each aquarium. At the end of a 24-h trial, a division was inserted to separate the habitats, and the numbers of megalopae and J1 crabs in each habitat were counted. The null hypothesis that neither type of habitat was preferred by megalopae was tested using a Wilcoxon test (Conover 1971).

Metamorphosis and cannibalism

This experiment was designed to test whether newly settled J1 crabs affect survival of megalopae molting next. Two sets of experimental treatments were run in 40-L aquaria where megalopae were placed at a density of $5 \cdot L^{-1}$: (1) Newly metamorphosed J1 crabs were removed from the aquaria every 4 h and held individually to the end of 96 h trials ($N = 5$). Crabs were daily monitored for survival. (2) Newly metamorphosed J1 crabs were not removed from the aquaria, and numbers of megalopae and J1 crabs were recorded every 24 h for 96 h ($N = 10$).

All crabs were fed with shrimp flakes during the 96-h test. Estuarine water was changed every 24 h and held constant at 15°C. The null hypotheses of no difference in the proportion of megalopae that did not metamorphose or in the proportion of J1 crabs that survived to 96 h between treatments were tested using a Mann–Whitney test (Zar 1984).

Cannibalism

This experiment was designed to evaluate whether J1 and J2 crabs cannibalize megalopae or newly settled J1. Cannibal (crab) density used in this experiment was comparable with that in the field shortly after settlement (approximately 300 crabs$\cdot m^{-2}$). Aquaria (10 L) were conditioned with a 5-cm layer of mud collected in the intertidal zone and a layer of oyster shells and were filled with estuarine water. Ten crabs and 10 megalopae were added per tank. Control tanks contained only megalopae. Twelve trials were run for each cannibal (crab) instar stage. After 24 h, surviving megalopae and newly molted J1 crabs were counted and the proportion of crab consumed estimated. A t-test was used to test the null hypothesis of no difference in the mean number of megalopae/J1 crabs eaten by J1 or J2 predators.

Results

Field Observations

No crabs of the 1990 year-class (CW > 25 mm) remained at plot SC-O by May 1991 (Fig. 2). The first cohort of megalopae settled between May 15 and 20 at plots SC-O and PC-O, at respective crab density ranges of 140-220 and 150-320 crabs$\cdot m^{-2}$ (Fig. 2).

The second cohort of megalopae settled between June 15 and 18 just after construction of the two unoccupied shell plots (SC-U and PC-U). Mean density of megalopae/J1 crabs (second cohort) remaining in the shell plots by late June was significantly higher at unoccupied than at occupied plots (ANOVA: $F = 277.86$, df = 1,1, $P = 0.038$; Fig. 3) but was not significantly different between sites (Pacman and South Channel; ANOVA: $F = 31.34$, df = 1,1, $P = 0.111$). Mean density of J2/J3 crabs (first cohort) was significantly higher in the occupied than in the

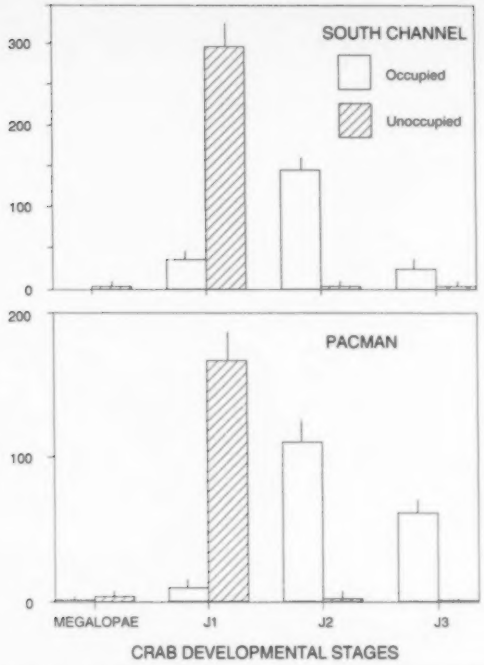

FIG. 3. Mean density of megalopae, J1, J2, and J3 Dungeness crabs in "occupied" (open bars) and "unoccupied" (hatched bars) shortly after the second peak of megalopae (June 26 and 27). Bars indicate mean density; lines indicate 1 SE. J1, first instar crab; J2, second instar crab; J3, third instar crab.

unoccupied shell plots (ANOVA: $F = 395.02$, df = 1,1, $P = 0.032$; Fig. 3), consistent with settlement a month earlier, but not significantly different between sites (ANOVA: $F = 1.43$, df = 1,1, $P = 0.44$). There were low densities of J2 and J3 crabs in the unoccupied shell plots (Fig. 3), probably as a consequence of immigration from surrounding areas.

Laboratory Experiments

Shell selection

Habitat selection experiments under laboratory conditions showed that Dungeness crab megalopae preferred weathered (mean proportion = 0.63, SD = 0.15, $N = 10$) over clean shell ($Z = 2.12$, $N = 10$, $P = 0.034$).

Metamorphosis and cannibalism

Most megalopae had settled and metamorphosed by the end of 96-h trials, but a significantly higher proportion of the initial number of megalopae remained when J1 crabs were allowed to accumulate (treatment 2; $X = 0.12$, SD = 0.06, $N = 10$) compared with tanks where J1 crabs were removed (treatment 1; $X = 0.07$, SD = 0.01, $N = 5$; $U = 45$, df = 5,10, $P < 0.01$; Fig. 4).

The mean proportion of J1 crabs that survived the 96-h experimental period after molting was higher in treatment 1 (removed and held separately) than in treatment 2 ($U = 0$, df = 5,10, $P < 0.01$). All crabs from treatment 1 held in individual holding tanks survived the 96-h experiment.

Cannibalism

Crabs were observed preying on megalopae. J2 consumed significantly more megalopae (mean number of megalopae/J1 eaten = 9.83, SD = 0.57, $N = 12$) than J1 crabs (mean number of megalopae/J1 eaten = 4.0, SD = 1.12, $N = 12$; t-test = –15.94, df = 22, $P < 0.0001$). Loss of megalopae in control aquaria was low (mean number of megalopae/J1 lost = 0.8, SD = 0.9, $N = 12$).

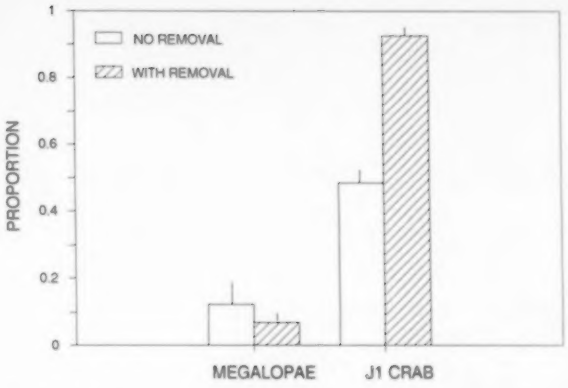

FIG. 4. Mean proportion of Dungeness megalopae that did not metamorphose to J1 instar and mean proportion of J1 crabs, that molted and survived during the 96-h experiment in both treatments. Open bars: no J1 crabs were removed during the 96 h test; hatched bars: J1 crabs were removed every 4 h and held individually to 96 h.

Discussion

Field data show that the settlement of the second cohort of megalopae and/or survival of J1 crabs in shell habitats occupied by the first cohort were low; density of J1 crabs was only 5–10% of that found in unoccupied shell habitats. The difference is not due to preference by Dungeness crab megalopae for new clean shell over weathered shell, since under laboratory conditions, megalopae selected the latter. If shell habitat were selected similarly in nature, the opposite pattern of J1 density in June would be expected. We suggest three alternative hypotheses to explain the pattern of J1 crab density observed between occupied and unoccupied shell habitats: (a) cannibalism, (b) conspecific avoidance, and (c) post-settlement competition.

(a) Cannibalism

The first cohort of juvenile crabs cannibalized successive cohorts of megalopae and/or J1 that settled in occupied intertidal shell plots. This hypothesis is supported by our laboratory results which suggest that cannibalism between cohorts of 0+ Dungeness crabs may be an important determinant of population abundance and structure. In addition, laboratory experiments also show that cannibalism within a single cohort probably occurs, since 40% fewer J1 crabs survived 96-h tests when held *in mass* after molting from megalopae than when held individually (Fig. 4). Cannibalism is common in many species (Fox 1975; Polis 1981) and can be a major factor influencing population structure, life history, and behavior (Polis 1981). Although cannibalism is also a common phenomenon among decapod crustaceans (Gotshall 1977; Jamieson et al. 1982; Hines et al. 1990; Fitz and Wiegert 1991), its role in structuring natural populations has been largely overlooked (but see Botsford and Wickham 1978). Cannibalism on newly settled YOY recruits by older age-classes has been reported in several field studies of

brachyuran crabs (Gotshall 1977; Kurihara and Okamoto 1987; Kurihara et al. 1988; Fitz and Wiegert 1991) and lobsters (Jamieson et al. 1982), but it has only occasionally been reported between YOY siblings. Reise (1985) suggested that cannibalism among YOY sibling shore crabs, *Carcinus maenas*, may have caused declines in their abundance during field experiments. Under laboratory conditions, cannibalism among YOY has been reported for the Caribbean king crab, *Mithrax spinosissimus* (Tunberg and Creswell 1988), shore crab (Klein Breteler 1975), and Dungeness crab (Jacoby 1983; Karpov 1983). Cannibalism among YOY based on stomach contents may be difficult to detect in Dungeness crabs since YOY crabs do not consume the carapaces and appendages of their conspecifics (M. Fernandez, unpubl. data).

Since settlement of several cohorts of Dungeness megalopae during summer appears to be the norm (Orensanz and Gallucci 1988; McConnaughey et al. 1992; Dinnel et al. 1993), cannibalism among YOY crabs may be an important factor in population dynamics of this species, dependent on the relative strength of different cohorts and the extent of spatial overlap, particularly within limited habitat such as intertidal shell (Dumbauld et al. 1993). Although we do not postulate intercohort competition, the advantages to larger individuals of an earlier cohort that cannibalize smaller conspecifics are to reduce competition for limited space and food exacerbated by later cohorts that settle at densities up to 300 crabs$\cdot m^{-2}$ and, simultaneously, to utilize the small J1 crabs as prey to meet short-term energy requirements that might be in excess of production of other prey within shell habitats (e.g., small bivalves, polychaetes, and amphipods).

(b) Conspecific Avoidance

Settling megalopae avoid conspecifics or some other species inhabiting occupied shell plots. Dungeness megalopae may have chemically or tactilely detected older 0+ conspecifics, delayed their metamorphic molt as they did under laboratory conditions, and moved on later tides to other habitats. Although settlement avoidance in response to conspecifics has not been reported for Dungeness crabs or other brachyuran crabs, the opposite pattern has been shown for porcelain crab megalopae (Jensen 1991). Megalopae of *Petrolisthes cinctipes* and *Petrolisthes eriomerus* settle in response to conspecific adults, probably using a waterborne cue (Jensen 1991). If Dungeness crab megalopae have the capability to detect older postsettlement conspecifics, avoidance behavior is advantageous only if they are able to find other suitable benthic habitat not yet occupied or with lower conspecific density to ensure a good chance of survival. If instead all suitable habitat had been occupied by the first cohort, survival rates of subsequent cohorts are assumably low because of prolonged planktonic life and lower postsettlement survival in more exposed locations (Fernandez et al. 1993).

(c) Postsettlement Competition

Crabs of the second cohort may have failed to recruit in occupied plots because space in the shell habit was physically saturated by crabs of the first cohort. Since small instars are highly agonistic in encounters (Jacoby 1983; personal observation), small J1 crabs may be driven to the surface of the shell matrix or to open tideflats where predation is high (Fernandez et al. 1993). Juvenile crabs (J3 and J4) abandon artificial shell habitats in response to high density of conspecifics, although food is not a limiting resource, and face high predation risk on the open mud (personal observation).

Presently we are unable to reject any hypotheses but suggest, on the basis of laboratory observations and experiments, that larger crabs of the May cohort may have cannibalized megalopae and J1 crabs of the June cohort in the occupied shell habitats.

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Determinants of Postmolt Size in the American Lobster (*Homarus americanus*). I. D_1^3 is the Critical Stage

Jin-Hua Cheng[1] and Ernest S. Chang[2]

Bodega Marine Laboratory, University of California, P.O. Box 247, Bodega Bay, CA 94923, USA

Cheng, J.-H., and E.S. Chang. 1993. Determinants of postmolt size in the American lobster (*Homarus americanus*). I. D_1^3 is the critical stage. Can. J. Fish. Aquat. Sci. 50: 2106–2111.

Juvenile American lobster (*Homarus americanus*) were subjected to induced limb autotomy, eyestalk ablation, and manipulation of container space at different molt stages to test the hypothesis that postmolt size is predetermined at the time of deposition of the epicuticle. Molt increments in autotomized animals that regenerated limbs were reduced by 30–40%. Molt increments were reduced by only 5–10% in autotomized animals that did not regenerate limbs. The critical stage for limb regeneration is D_1^3. Depending on the time of limb autotomy, molt intervals were decreased (stages B–C), increased (stages D_1^1 and D_1^2), or not affected (stages D_0, D_1^3, D_2, and D_3). Eyestalk ablation shortened the molt interval; the change in molt interval was less when ablation was performed near the end of the current molt cycle. Eyestalk-ablated lobsters had larger molt increments compared with intact controls, especially in the chelipeds. This growth-promoting effect disappeared if ablation was conducted at or after D_1^3. Transferring lobsters from small to large container spaces shortened molt intervals and increased molt increments. Increases in molt increments only occurred when transfers were made before D_1^3. We conclude that postmolt size of lobsters is predetermined at D_1^3 when the new epicuticle is produced.

Des homards (*Homarus americanus*) juvéniles ont été soumis à une autotomie induite des membres, une ablation du pédoncule oculaire et une manipulation de l'espace dans le vivier à différents stades de la mue; nous voulions vérifier l'hypothèse selon laquelle la taille après la mue serait prédéterminée au moment de la tombée de l'épicuticule. L'accroissement à la mue chez les animaux autotomisés dont les membres se régénéraient était réduit de 30–40 %. Cet accroissement était réduit de seulement 5–10 % chez les animaux autotomisés sans régénération. Le stade critique pour la régénération des membres est D_1^3. Selon le moment de l'autotomie, l'intervalle entre les mues diminuait (stades B–C), s'accroissait (stades D_1^1 et D_1^2) ou n'était pas affecté (stades D_0, D_1^3, D_2 et D_3). L'ablation du pédoncule oculaire raccourcissait l'intervalle entre les mues; le changement de cet intervalle était moindre quand l'ablation avait lieu près de la fin du cycle de mue en cours. Les homards ayant subi l'ablation du pédoncule oculaire présentaient des accroissements à la mue supérieurs à ceux des sujets témoins intacts, particulièrement dans les chélipèdes. Cet effet favorable à la croissance disparaissait si l'ablation avait lieu au stade D_1^3 ou après. Le transfert des homards de viviers de petite taille à des viviers de grande taille raccourcissait l'intervalle des mues et augmentait l'accroissement à la mue. Cette augmentation se produisait seulement lorsque les transferts étaient effectués avant D_1^3. Nous en concluons que la taille après la mue chez les homards est prédéterminée à D_1^3, au moment de la production du nouvel épicuticule.

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The lives of crustaceans, like those of other arthropods, revolve around the cyclic need to deposit a new cuticle and to shed the old one. This cyclic event (molting) is a prerequisite to the appearance of changes in body size and shape.

Mole increment, the percentage increase in size that occurs between one instar and the next, is extremely variable both among and within species. Values ranging from a high of 83% to a low of less than zero have been noted in various species. Within a particular species, molt increments vary according to various factors such as size, sex, limb regeneration, food supply, and eyestalk ablation (Aiken 1980; Hartnoll 1982).

The mechanisms that might control molt increment have been studied little in either crustaceans or insects. It has been suggested that size increase at molt is programmed by the increase in cell number at stages D_0–D_1 (Kuris and Mager 1975). Alternatively, in eyestalk-ablated larval shrimp, increased postmolt size was attributed to extension of the soft cuticle (Okazaki and Freeman 1993). However, we believe that it is the size of the premolt cuticle, a product of the epidermal cells, that delimits postmolt size (Bennet-Clark 1963; Reynolds 1980; J.-H. Cheng and E.S. Chang, unpublished data).

In *Rhodnius prolixus*, molt increment is limited by the size of the epicuticle, which is inextensible (Bennet-Clark 1963; Filshie 1976, 1982). It is the first layer of cuticle deposited (Wigglesworth 1973). In addition, size of the abdomen in the adult is directly related to its size in the larva at the time of epicuticle deposition (Bennet-Clark 1971). In Atlantic sand fiddler (*Uca pugilator*) the growth-promoting effect of eyestalk ablation only occurred when the operation was performed at least 6 d before molt (Passano 1953); this is approximately the time of deposition of the new cuticle. The above results support the hypothesis that size increase at molt is predetermined at the time of deposition of the epicuticle.

[1]Present address: Tungkang Marine Laboratory, Tungkang, Pingtung, Taiwan 928.

[2]Author to whom all correspondence should be addressed.

Autotomy of appendages before the critical stage allows limb regeneration to occur before molt. This results in a lengthening of the molt interval and a reduction of the molt increment in other body parts (Skinner 1985). Therefore, the critical stage for regeneration may represent the critical stage for resetting not only the molt interval (Hoarau and Hirn 1981; Hopkins 1982), but also the molt increment.

In this study, the hypothesis that postmolt size is predetermined at the time of epicuticle deposition was tested. Three treatments, induced limb autotomy, eyestalk ablation, and space manipulation, were made on juvenile American lobsters (*Homarus americanus*) at different molt stages. In addition, the critical stage for autotomy to increase the molt interval and that for eyestalk ablation to decrease it were determined.

Methods

Maintenance of Animals

Sibling juvenile lobsters (5–10 g wet weight, 19.6–24.5 mm carapace length, and 57.4–71.6 mm body length) were raised in a semirecirculating system at 20.0 ± 0.5°C and a 16 h light: 8 h dark photoperiod (Chang and Conklin 1993; Conklin and Chang 1993). They were fed a live adult brine shrimp diet.

Molt Stage Determination

Staging of postmolt and early intermolt was determined according to the degree of softness of the carapace and chelae as reported by Stevenson (1968). Stage B lobsters were somewhat brittle, except for the postorbital ridge and the cervical groove. Stage C began when this ridge and groove became rigid.

Premolt juvenile lobsters were staged based on setal development on the pleopods (Aiken 1973). The progress of setal development was examined in situ under a dissecting microscope, and six different premolt stages were determined. Stage D_0 was characterized by epidermal retraction in the pleopods. Stage D_1^1 was characterized by the appearance of scalloped epidermis between the future setae. Stage D_1^2 was characterized by epidermal invaginations at the future setae, but without the appearance of setal shafts. At stage D_1^3, shafts of the setae were visible, but the proximal ends were not well defined. At stage D_2, the ends of the setal shafts were visible and bifurcated. Stage D_3 was characterized by pleopods with blunt setal shafts that were thick and dark.

Treatments

Lobsters grouped by molt stages were subjected to three different treatments to investigate the critical molt stage that determined postmolt size. The treatments used were induced limb autotomy, eyestalk ablation, and container space manipulation. For limb autotomy, lobsters were induced to autotomize both of their chelipeds by applying constant pressure to each merus with forceps. For eyestalk ablation, both eyestalks were removed at their bases from intact animals using a pair of fine scissors. Survival was approximately 90%. For container space manipulation, lobsters were transferred from large (190 × 190 mm) to small (95 × 95 mm) square containers or vice versa. Except for the container space transfer experiment, lobsters were raised individually in the small containers.

For each experiment, trays containing the lobsters were kept in the same culture system at a depth of 80 mm. All animals were fed an excess amount of food during the experimental period.

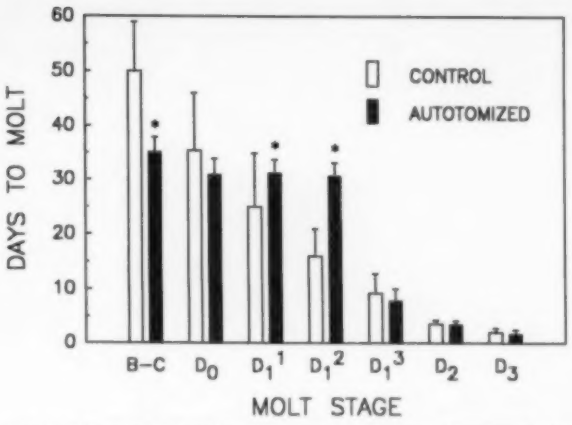

FIG. 1. Effects of induced autotomy on molt interval. Number of days to molt following autotomy is plotted against the molt stage when lobsters were induced to autotomize both chelipeds. Asterisks indicate significant differences from intact control lobsters.

Molt Increment

Lobsters were checked for molt every day. Carapace length (CL) and cheliped (propus) width (ChW) of each lobster were measured to the closest 10 μm using calipers both before treatment and 1 wk after molt. The percentage increase in these parameters was calculated based on the following equation: $[(X_a/X_b) - 1] \times 100$, where X_a and X_b are either CL or ChW measured from the same lobster after and before molt, respectively. Amount of allometric growth in width of the cheliped was calculated based on the following: the allometric ratio equals the percentage increase in cheliped width divided by the percentage increase in carapace length. Statistical analyses were performed using the SAS-GLM program (SAS Institute Inc.) designed for the analysis of variance with uneven sample sizes. The comparison among molt stages within treatment groups was made using Duncan's multiple range comparison test. All comparisons were made at a significance level of 0.05. In the figures, mean values with the same letters are not significantly different ($p > 0.05$). All values are expressed as means ± 1 SD. Each group contained at least seven animals.

Results

Depending on the molt stage when their chelipeds were autotomized, lobsters may regenerate a limb bud before the next molt. If chelipeds were removed before D_1^3, they regenerated limb buds before the subsequent molt; otherwise they did not. Molt interval was strongly affected by regeneration (Fig. 1). Compared with intact controls, the molt interval decreased if cheliped autotomy occurred at B–C (postmolt to intermolt) and increased if autotomy occurred at D_1^1 and D_1^2 (just prior to the critical stage D_1^3). Molt interval was not affected if autotomy occurred at the beginning of premolt (D_0) or at late premolt (D_1^3–D_3). All lobsters with limb buds completed regeneration in about the same amount of time (mean of 31–35 d for all groups) after the loss of their chelipeds (Fig. 1). There were no differences in the molt intervals of lobsters that autotomized during D_0, D_1^1, or D_1^2. The molt intervals of lobsters that autotomized during B–C required only four additional days to complete regeneration and molt. The smaller variations in molt interval

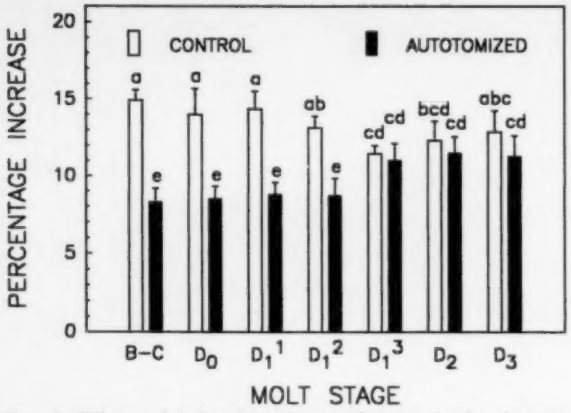

FIG. 2. Effects of induced autotomy of postmolt size increase. Percentage increase in postmolt carapace length is plotted against the molt stage when lobsters were induced to autotomize both chelipeds. Chelipeds were regenerated before molt in lobsters autotomized in stages B–C, D_0, D_1^1, and D_1^2 but not in stages D_1^3, D_2, and D_3.

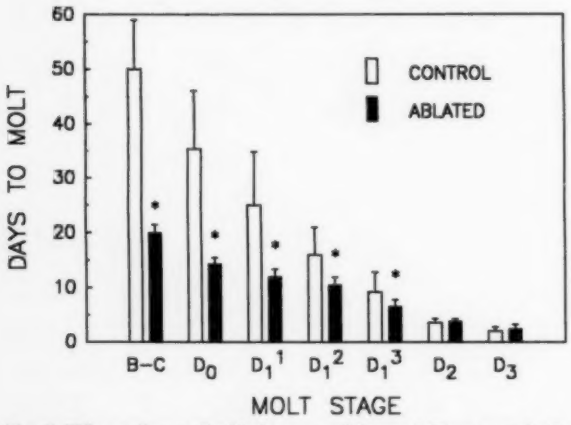

FIG. 3. Effects of eyestalk ablation on molt interval. Days to molt after ablation is plotted against the molt stage when eyestalks were ablated. Asterisks indicate significant differences from intact controls.

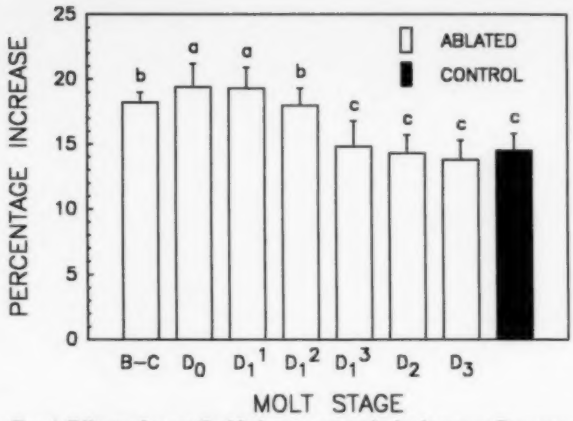

FIG. 4. Effects of eyestalk ablation on postmolt size increase. Percentage increase in carapace length after molt is plotted against the molt stage when eyestalks were ablated.

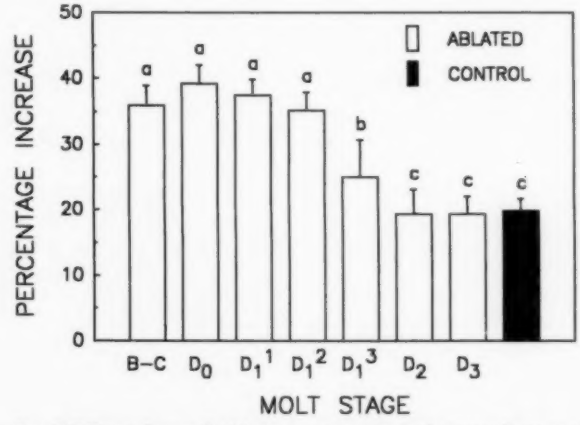

FIG. 5. Effects of eyestalk ablation on postmolt size increase. Percentage increase in postmolt cheliped (propus) widths is plotted against the molt stage when eyestalks were ablated.

observed in autotomized lobsters indicated a greater synchronization in molting compared with intact lobsters (Fig. 1).

Regeneration affected not only the molt interval but also the postmolt size increase. Figure 2 shows the percentage increase in postmolt carapace length (%CL) of intact and cheliped-autotomized lobsters. When compared with intact controls, regenerating lobsters (autotomized in B–D_1^2) showed a 30–40% reduction in %CL. Nonregenerating lobsters (animals autotomized in D_1^3–D_3, which did not grow limb buds) showed only a nonsignificant reduction (5–10%) in %CL.

Eyestalk ablation shortened the molt interval (Fig. 3). This effect was diminished in lobsters ablated nearer the normal time of molt; the effect became insignificant when animals reached D_2. Eyestalk ablation also affected %CL (Fig. 4). Compared with intact controls, an eyestalk-ablated lobster had a higher %CL if both eyestalks were removed before D_1^3. The effects were greater if ablation occurred in D_0 and D_1^1 compared with B–C and D_1^2. This growth-promoting effect did not occur in lobsters that had their eyestalks removed at D_1^3 or later.

Eyestalk removal before D_1^3 promoted growth not only in carapace length but also in cheliped width (Fig. 5). Interestingly, the effect was much more pronounced in the percentage increase in postmolt cheliped width (%ChW) than in %CL. Compared with intact lobsters, %ChW was about 80% larger whereas %CL was only about 30% larger in eyestalk-ablated lobsters (Fig. 4). A partial effect was seen in %ChW but not in %CL in lobsters whose eyestalks were removed at D_1^3.

The ratio %ChW to %CL, an index of allometric growth, is shown in Fig. 6. If eyestalk ablation was conducted before D_1^3, a ratio of about 2.0 resulted. If ablation was conducted after D_1^3, a ratio of about 1.4 was observed, similar to that seen in an intact lobster. Eyestalk ablation conducted at D_1^3 resulted in a lobster with an intermediate value (about 1.7).

Molt intervals were decreased if lobsters were transferred from small to large container spaces (Fig. 7). This effect became less pronounced as lobsters approached the end of the molt cycle and became nonsignificant when they reached D_1^2. Figure 8 shows the effect of container space on the %CL. Compared with

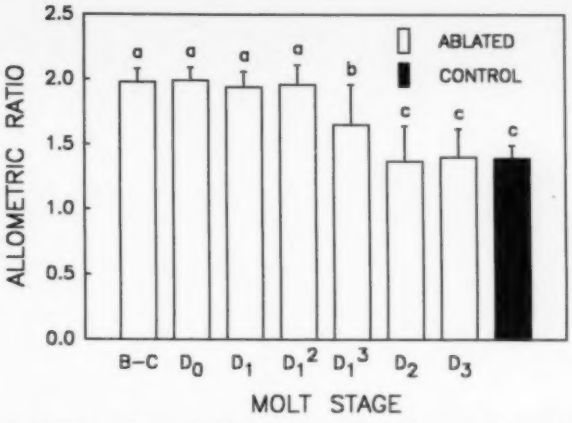

FIG. 6. Effects of eyestalk ablation on postmolt size increase. Allometric growth in cheliped widths is calculated from data in Fig. 4 and 5, based on the equation described in the text.

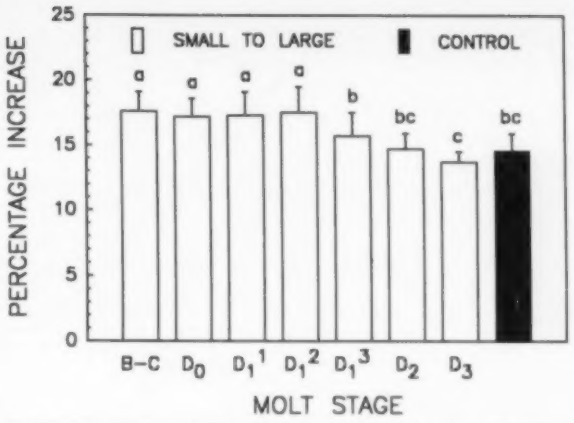

FIG. 8. Effects of container space on postmolt size increase. Percentage increase in postmolt carapace length following transfer from small to large containers is plotted against the molt stage when lobsters were transferred. Control lobsters were raised continuously in small containers.

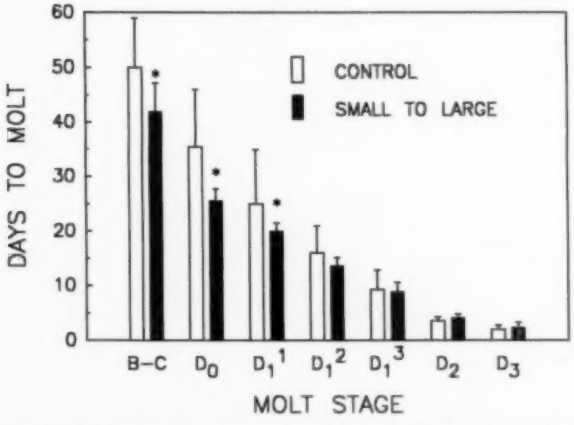

FIG. 7. Effects of container space on molt interval. The number of days to molt following transfer from small to large containers is plotted against the molt stage when lobsters were transferred. Asterisks indicate significant differences.

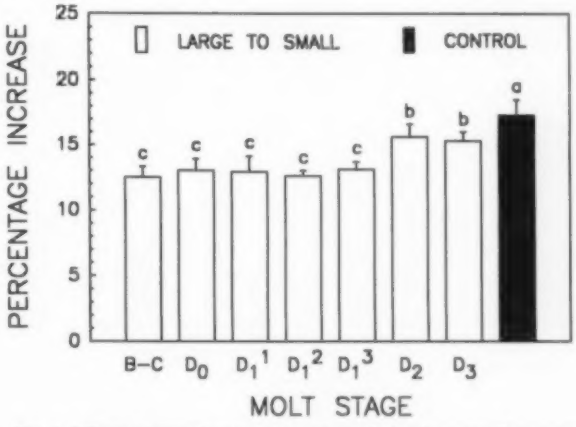

FIG. 9. Effects of container space on postmolt size increase. Percentage increase in postmolt carapace length following transfer from large to small containers is plotted against the molt stage when lobsters were transferred. Control lobsters were raised continuously in large containers.

control lobsters that were raised continuously in small containers, animals transferred from small to large containers before D_1^3 showed significant increases in %CL. No increases in %CL were observed if the transfers were made at D_1^3 or later. In contrast, lobsters transferred from large containers to small ones showed decreases in %CL (Fig. 9) if compared with the control animals that were raised continuously in large containers. The decreases were smaller if transfers were made after D_1^3.

Discussion

The relationship between molting and regeneration has been a subject of extensive study in both insects and crustaceans (see Bulliere and Bulliere 1985 and Skinner 1985 for reviews). In both taxonomic groups, there are critical stages that permit regeneration prior to the first postoperative molt. In this study, we showed that the critical stage for regeneration in juvenile lobsters is D_1^3. If chelipeds were autotomized before this critical stage, they were regenerated at the first postoperative molt. Otherwise, they were not regenerated until the completion of the second postoperative molt. This observation is consistent with data from crabs (Skinner and Graham 1972; Charmantier-Daures 1976; Hopkins 1982) and an isopod (Hoarau and Hirn 1981). In those species, however, D_1 was the critical stage. The staging method of Aiken (1973) allowed us to pinpoint D_1^3 as the critical stage.

In those lobsters that were autotomized and regenerated chelipeds in the same molt cycle, regeneration took about the same amount of time to complete, regardless of the molt stage when they underwent autotomy. Therefore the molt interval was decreased or increased, depending on the stage in which autotomy occurred. For example, a decreased molt interval occurred when chelipeds were removed at the beginning of the molt cycle;

an increased molt interval occurred when autotomy was conducted at stages approaching the critical stage. Similar results have also been reported in crabs (Skinner and Graham 1972; Charmantier-Daures 1976; Holland and Skinner 1976; Hopkins 1982). The observation that the amount of time required for regenerating lobsters to complete regeneration and molting was as long as a normal premolt period in intact lobsters supports the hypothesis that the effect of autotomy is to reinitiate a normal sequence of premolt events (Noarau and Hirn 1981; Hopkins 1982).

It appears that loss of chelipeds before D_1^3, when the epicuticle is deposited (Aiken 1980), is a signal for lobsters to reschedule the molt cycle, thus permitting regeneration. Also, cheliped loss before deposition of the epicuticle allows the lobster to reprogram its molt increment to compensate for the extra growth needed for regeneration. The degree of reduced growth in other body parts in response to the need for regeneration depends on the total mass of the lost appendages (Skinner and Graham 1972; Kuris and Mager 1975; Hopkins 1982). The concept of regeneration load has been applied to this phenomenon (Skinner and Graham 1972). Reduction in %CL in regenerated crustaceans is not due to redistribution of water (Hopkins 1985), but instead to redistribution of energy available for growth (Kuris and Mager 1975). In fact, this growth-depressing effect can be explained by the smaller size of the new cuticle made by regenerating animals (J.-H. Cheng and E.S. Chang, unpublished data). The observations that regeneration reduces the size increase of other body parts and that regeneration occurs only before D_1^3 are consistent with the hypothesis that size increase at molt is predetermined at time of epicuticle deposition.

In lobsters, as in many other crustaceans, eyestalk ablation results in an increase in %CL. In our study, this effect did not occur if the operations were performed at or after D_1^3. A similar phenomenon was observed by Passano (1953). He found that the growth-promoting effect of eyestalk ablation in *U. pugilator* was dramatically reduced if the operation was conducted during late premolt. Based on that result, Passano (1960) suggested that the cuticle made by eyestalk-ablated crabs was more elastic than that of intact ones. Our data do not support that suggestion in lobsters, since this growth-promoting effect can be explained by the larger new cuticle made by eyestalk-ablated animals (J.-H. Cheng and E.S. Chang, unpublished data). The result that the growth-promoting effects of eyestalk ablation cannot be expressed after epicuticle is laid down is also consistent with our hypothesis that postmolt size is predetermined at the time of epicuticle deposition.

Space availability affects not only molt interval but also molt increment, as seen in many other crustaceans (Aiken and Waddy 1978; Aiken 1980; Hartnoll 1982). In this study, the space factor (surface area of each container divided by the square of the carapace length) was 15.2–23.4 and 60.8–92.2 for the small and large containers, respectively. Lobsters raised in small containers had smaller %CLs than those raised in large ones. Similar results were obtained from lobsters between 60 and 80 mm CL, where space factors of 18 and 55 resulted in %CLs of 10.3 and 14.3, respectively (Aiken and Waddy 1978).

Molt stage affects the container space effect on molt increment. Templeman (1948) studied juvenile *H. americanus* and found that transferring lobsters to larger containers increased the %Cl. This increase did not occur if lobsters were transferred shortly before molt, but occurred at the subsequent molt when lobsters resided in larger containers for a complete molt cycle. He believed that acclimation time was the necessary variable that permitted lobsters to adjust to a new environment. The results of our study indicate that transferring lobsters from small to large environments increases %CL as long as the transfers were made before the critical stage D_1^3, further supporting our hypothesis that molt increment is predetermined when the epicuticle is laid down.

Eyestalk ablation affects not only the size, but also the shape of lobsters after molt. In eyestalk-ablated animals, the %ChW is larger than the %CL. The ratio of %ChW to %CL is about 2.0 in eyestalk-ablated lobsters compared with 1.4 in intact ones. The observation that changes in allometric growth of the chelipeds disappear if the eyestalks are removed after D_1^3 supports the hypothesis that both postmolt shape and size are determined at the time of epicuticle deposition. The reason for the partial effect observed in the chelipeds at D_1^3 is not clear. It is possible that the epicuticle is laid down later in the chelipeds than in the carapace. The observation that deposition of epicuticle in the pleopods begins later at D_2 instead of D_1^3 (Aiken 1973) provides evidence for differential cuticle deposition.

Eyestalk ablation has also been shown to have effects on morphogenesis at the critical molts associated with the larval–juvenile transition (metamorphosis) in crabs (Costlow 1966a, 1966b), shrimps (Le Roux 1984, 1988), and lobsters (Snyder and Chang 1986; Charmantier et al. 1988) and the juvenile–adult transition in female crabs (H. Laufer, personal communication). The effects of eyestalk ablation on morphogenesis associated with critical molts only occurred when eyestalks were removed before a critical stage. In both lobster (Charmantier et al. 1988) and shrimp (Le Roux 1988) larvae, the critical stage was D_1^3. These results add further support to the hypothesis that postmolt shape and size are predetermined at the time of deposition of the epicuticle.

Judging from the results that the growth-promoting effects of eyestalk ablation and transfer into a larger container space cannot be expressed if the eyestalk ablations or transfers were made after D_1^3, it appears that molt increment is unable to increase after the deposition of the epicuticle. The reduction in molt increment seen in both autotomized and intact lobsters, however, shows that molt increment can be reduced even after the deposition of the epicuticle. A 10–12% reduction in molt increment was found in lobsters transferred to smaller container spaces after D_1^3. This reduction in increment is much smaller than the reductions seen in lobsters transferred at earlier molt stages (24–28%, Fig. 9). The latter observation agrees with capture data in adult lobsters. Molt increments were much higher in adult lobsters captured and impounded (and therefore transferred to a smaller container space) a few days before molt compared with those lobsters caught several weeks before molt (Templeman 1948). Also, reduction of molt increment during late premolt was reported in rock lobsters (*Panulirus cygnus*). Molt increment was decreased if lobsters were handled within 2 wk of molt (Chittleborough 1974). The mechanism responsible for that particular late premolt reduction in molt increment is not known.

Acknowledgments

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Department of Commerce, under grant NA89AA-D-SG138, project R/A-80, through the California Sea Grant College, and by the California State Resources Agency (to E.S.C.). The views expressed herein are those of the authors and do not necessarily reflect the views of NOAA or any of its sub-agencies.

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Phylogenetic Relationships among Members of the Coregoninae Inferred from Direct Sequencing of PCR-Amplified Mitochondrial DNA

S. Fournier Lockwood

Bioindustrial Technologies, Inc., 40200 Industrial Park Circle, Georgetown, TX 78626, USA

Robert E. Dillinger Jr.

Idaho Department of Fish and Game, 1798 Trout Rd., Eagle, ID 83616, USA

Tim P. Birt

Department of Ornithology, Royal Ontario Museum, 100 Queen's Park, Toronto, ON M5S 2C6, Canada

John M. Green

Department of Biology, Memorial University of Newfoundland, St. John's, NF A1B 3X9, Canada

and Thomas P. Snyder

Department of Biology, Michigan Technological University, Houghton, MI 49931, USA

Lockwood, S.F., R.E. Dillinger Jr., T.P. Birt, J.M. Green, and T.P. Snyder. 1993. Phylogenetic relationships among members of the Coregoninae inferred from direct sequencing of PCR-amplified mitochondrial DNA. Can. J. Fish. Aquat. Sci. 50: 2112–2118.

We determined the DNA sequence of a portion of the mitochondrial cytochrome *b* gene for eight members of the Coregoninae (Salmonidae) from North America in an effort to elucidate phylogenetic relationships in the subfamily. DNA was prepared for sequencing by the polymerase chain reaction (PCR). Direct estimates of mitochondrial DNA (mtDNA) sequence divergence among taxa ranged from 0.0% between Arctic cisco (*Coregonus autumnalis*) from the Mackenzie River, Canada, and cisco (*C. artedi*) from the Laurentian Great Lakes to 5.8% between the inconnu (*Stenodus leucichthys*) and the round whitefish (*Prosopium cylindraceum*). As has been noted in other fish species and vertebrates in general, third position silent substitutions predominated over any other type of nucleotide change. No amino acid replacement substitutions were found among any of the eight taxa examined. Comparison of mtDNA sequence divergence estimates from this and other studies suggests that the radiation of the genera within the Coregoninae occurred relatively recently (2–6 million yr B.P.). Cladistic analysis of the mtDNA sequence data yields a hypothesis of relationships that supports previous genetic and morphological classifications of coregonines.

Nous avons déterminé la séquence d'ADN pour une portion du gène du cytochrome *b* mitochondrial chez huit membres des Coregoninae (Salmonidae) d'Amérique du Nord, afin de tenter de mettre en lumière les relations phylogénétiques qui existent dans cette sous-famille. Nous avons préparé l'ADN pour le séquençage par la réaction de polymérisation en chaîne. Les estimations directes de la divergence dans les séquences d'ADN mitochondrial entre les taxons allaient de 0,0 % entre le cisco arctique (*Coregonus autumnalis*) du Mackenzie, au Canada, et le cisco de lac (*C. artedi*) des Grands Lacs Laurentiens, à 5,8 % entre l'inconnu (*Stenodus leucichthys*) et le ménomini rond (*Prosopium cylindraceum*). Comme cela a été noté chez d'autres espèces de poissons et de vertébrés en général, les substitutions muettes de troisième position prédominaient par rapport à tous les autres types de changements dans les nucléotides. On n'a observé aucune substitution touchant les acides aminés parmi les huit taxons examinés. La comparaison des estimations sur la divergence des séquences d'ADN mitochondrial entre notre étude et d'autres semble indiquer que l'éloignement des genres au sein de la sous-famille Coregoninae ne s'est produit que relativement récemment (2 à 6 millions d'années avant notre ère). L'analyse cladistique des données sur les séquences d'ADN mitochondrial apporte une hypothèse sur les relations taxinomiques qui vient étayer des classifications antérieures, génétiques ou morphologiques, des corégoninés.

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The taxonomic relationships among the whitefishes and ciscoes (subfamily Coregoninae, family Salmonidae) are not clear. Most of the meristic and morphometric characteristics used as identifying features in other fish families are quite plastic in the coregonines and may undergo a great deal of environmental modification (Behnke 1972; Todd et al. 1981;

TABLE 1. Sources and numbers of coregonine fishes used in this study, and tissue type used for DNA amplification. Tissue designations; E, eggs; B, blood; L, liver; and K, kidney.

Species	Collection locality	*N*	Tissue type	Collection date
S. leucichthys	Arctic Red River, N.W.T., Canada	1	E	7-26-87
P. cylindraceum	Sagavanirktok River delta, Prudhoe Bay, Alaska	1	B	7-11-89
C. autumnalis	Liard River, B.C., Canada	1	E	9-14-88
	Peel River, N.W.T., Canada	1	B	7-22-88
C. artedi	Lake Superior, Baraga, Mich.	4	L	4-07-91
C. laurettae	Yukon River main stem, Ruby, Alaska	4	K	8-14-85
C. nasus	Sagavanirktok River delta, Prudhoe Bay, Alaska	1	B	8-08-89
C. pidschian	Sagavanirktok River delta, Prudhoe Bay, Alaska	1	B	7-18-89
C. sardinella	Sagavanirktok River delta, Prudhoe Bay, Alaska	1	B	7-10-89

Dillinger 1989). Additionally, some members are known to hybridize readily with one another (Berg 1948; Svardson 1970), and introgression is not an uncommon phenomenon (Smith 1964; Behnke 1972; Todd et al. 1981). The combination of phenotypic plasticity and convergence of morphological characters has resulted in multiple classifications of coregonines (reviewed in Smith and Todd 1993). The need for an evolutionary classification of coregonines, based upon characters that are largely independent of environmental influences, has been noted previously (Bernatchez et al. 1991). Both allozymes and mitochondrial DNA (mtDNA) fit such a description and have proven useful in studies of salmonid, and particularly coregonine, phylogeny (Ferguson et al. 1978; Allendorf et al. 1979; Grewe et al. 1990; Bodaly et al. 1991).

In this study, we examine the relationships of North American coregonines, inferred from mtDNA sequences. The rapid evolution of mtDNA and its maternal mode of inheritance, lack of recombination, and rapid sorting time provide possibly the highest resolution markers for delineation of matriarchal phylogenies of animals (Brown 1985; Avise et al. 1987; Birky et al. 1989). Recently, indirect sequencing of mtDNA has proven to be of great value in determining intra- and interspecific relationships within the Coregoninae (Bernatchez et al. 1988, 1989, 1991; Bernatchez and Dodson 1990a, 1990b) and salmonids in general (Wilson et al. 1987; Grewe et al. 1990). Such studies have used restriction enzyme fragment analysis of purified mtDNA, a relatively labor-intensive and time-consuming approach that is generally limited to closely related species due to the difficulty in aligning restriction maps among distantly related taxa (Wilson et al. 1985; Kocher et al. 1989).

We have used a polymerase chain reaction (PCR) amplification/direct sequencing approach (Carr and Marshall 1991), targeting an easily aligned, relatively conserved portion of a protein-coding gene of mtDNA (cytochrome *b*) whose primary structure, function, and pattern of evolution have been well characterized in vertebrates (Hatefi 1985; Irwin et al. 1991). These same sequences have proven valuable in addressing deep phylogenetic questions, such as the origin of tetrapods (Meyer and Wilson 1990). This study is an evaluation of the utility of the direct sequencing technique in elucidating phylogenetic relationships among closely related members of the Coregoninae. The following species are analyzed: inconnu (*Stenodus leucichthys*), least cisco (*Coregonus sardinella*), Arctic cisco (*C. autumnalis*), cisco (*C. artedi*), Bering cisco (*C. laurettae*), broad whitefish (*C. nasus*), humpback whitefish (*C. pidschian*), and round whitefish (*Prosopium cylindraceum*).

Materials and Methods

Localities and dates of collection, tissue types used for DNA extraction, and the number of individuals assayed for each species are listed in Table 1. Collections of *C. laurettae* and *C. autumnalis* were made during spawning runs of each species in their natal rivers; individuals were identified according to original species descriptions based on gill-raker counts (McPhail 1966; Table 1). This eliminated the confusion that persists in identifying Bering and Arctic cisco from coastal overwintering assemblages of these sibling species (Dillinger 1989). The single *P. cylindraceum* used in this study was a juvenile identified by parr marks and single nasal flap (Norden 1961). Voucher specimens of *P. cylindraceum*, *C. autumnalis*, *C. nasus*, *C. pidschian*, and *C. sardinella* were placed in the Texas Cooperative Wildlife Collection (TWCW), Texas A&M University, College Station, Texas.

Total genomic DNA was extracted from previously frozen (–80°C) tissues by digestion in 750 µL of a solution containing 100 mM Tris–HCl (pH 8.0), 10 mM EDTA, 100 mM NaCl, 0.1% SDS, 50 mM dithiothreitol, and proteinase K (0.5 µg/mL) for 2 h at 65°C. The DNA was purified by extracting twice with phenol, once with phenol–chloroform (1:1, v/v), and once with chloroform, precipitated with 2 volumes of absolute ethanol, and resuspended in 500 µL of distilled water. Purified mtDNA from *C. artedi* was prepared from fresh liver by ultracentrifugation (Lansman et al. 1981).

Double-stranded (symmetric) and single-stranded (asymmetric) PCR amplifications were performed using the cytochrome *b* primers L14841 and H15149 described by Kocher et al. (1989). This primer set amplified a 359-base-pair (bp) segment of cytochrome *b* from all fish examined. Reaction conditions for symmetric and asymmetric PCR and sequencing

TABLE 2. Matrix of number of transitions (above diagonal) and number of transversions (below diagonal) in pairwise comparisons among mitochondrial haplotypes for the eight coregonine species analyzed in this study. The mean number of transitions (5.6) was greater than the mean number of transversions (2.7) observed in 28 pairwise divergence comparisons, yielding a transition/transversion ratio of 2.07:1.

		1	2	3	4	5	6	7	8
1	*S. leucichthys*	—	8	7	7	9	10	9	12
2	*C. sardinella*	2	—	5	5	6	8	3	6
3	*C. autumnalis*	3	3	—	0	1	5	4	6
4	*C. artedi*	3	3	0	—	1	5	4	6
5	*C. laurettae*	3	3	2	2	—	6	5	6
6	*C. nasus*	2	2	4	4	3	—	5	6
7	*C. pidschian*	2	4	4	4	3	2	—	3
8	*P. cylindraceum*	2	4	3	3	3	2	0	—

were as described by Carr and Marshall (1991). Single-stranded amplification using dilutions of the heavy-strand primer was not routinely successful, as has been noted previously (Carr and Marshall 1991).

Sequences were aligned and prepared for publication with the ESEE program implemented by E. Cabot (Department of Biological Sciences, Simon Fraser University, Burnaby, B.C.). All sequences are given as their coding strand equivalents. A parsimony tree was constructed from character-state data using PAUP 3.0 (Swofford 1989). Confidence values for internal branches of the parsimony tree were assessed with the bootstrap option (Felsenstein 1985).

The direct sequencing approach described here has several distinct advantages over alternate analyses of mtDNA sequence divergence. PCR amplifications were routinely obtained from milligram amounts of a variety of tissues. Homologous sequences were aligned with ease relative to the restriction mapping approach, and with more confidence than fragment analysis, in which the homology of fragments and pattern of evolution of restriction sites are sometimes poorly understood (Wilson et al. 1985; Kocher et al. 1989). Phylogenetic information extending to the generic level was obtained in this study, taking into consideration the care that must be exercised when inferring phylogenetic relationships from short sequences of DNA (Martin et al. 1990). While it is likely that hypervariable sequences of the mtDNA molecule (such as the D-loop) will prove more useful in studies of postglacial dispersal within salmonid species (S.F. Lockwood, W.K. Thomas, and T.D. Kocher unpublished data), the relatively conserved cytochrome *b* sequences reported here may be ideally suited for interspecific and intergeneric comparisons.

Results

Figure 1 shows the aligned sequences of 241 bp of the coding strand of cytochrome *b* for the eight taxa surveyed. The 241 bp of sequence obtained represents approximately 1.5% of the 16 700-bp mitochondrial genome for these species, estimated from restriction enzyme fragment analysis (Bickham et al. 1989; Bernatchez et al. 1991). Twenty-three variable nucleotide positions were identified (comprising 9.5% of the 241 total sites) and were used in the subsequent phylogenetic analyses.

All mutations (transitions and transversions) were silent changes at third positions of codons (Fig. 1), underscoring the recent split of these lineages and the conservative rate of amino acid substitution noted previously in salmonid fish (Kocher et al.1989; Thomas and Beckenbach 1989). The mean number of transitions was greater than the mean number of transversions observed in 28 pairwise divergence comparisons, yielding a transition/transversion ratio of 2.07:1. The transition/transversion ratio reached a maximum of 6:1 in the pairwise comparison between *S. leucichthys* and *P. cylindraceum* (Table 2). These results reflect the general transition/transversion substitutional bias observed in the mitochondrial genomes of animals, which may exceed 10:1 in comparisons of closely related taxa (Brown 1985). Hence, the transition/transversion ratio of 2.07:1 observed in this study of coregonine fishes appears rather low by comparison.

No intraspecific sequence variation was uncovered for species with $N \geq 1$. Pairwise sequence divergence estimates ranged from 0.0% between *C. autumnalis* and *C. artedi* to a maximum of 5.8% between *S. leucichthys* and *P. cylindraceum* (Table 3).

Figure 2 shows the most parsimonious tree found by the exhaustive search option of PAUP, representing the variability among taxa in the cytochrome *b* gene with the fewest events. The cladogram was rooted with the primitive coregonine *P. cylindraceum*, as determined from morphological and genetic analyses (Bernatchez et al. 1988; Smith and Todd 1993). One tree of shortest length was found (28 steps); no other tree explained the variation more parsimoniously. The consistency index (CI) was 0.89, and the homoplasy index (HI) was 0.11. Only three sequence characters were inferred to have changed more than once. The consensus tree constructed after 100 bootstrap replications was identical to the shortest parsimony tree. All branches of the parsimony tree were found in greater than 50% of the 100 bootstrap replicates (branch and bound option of PAUP); branches that were supported strongly included that leading to *C. autumnalis, C. artedi,* and *C. laurettae* (87%) and that leading from *P. cylindraceum* to *C. pidschian* (78%).

The homologous sequence for pink salmon (*Oncorhynchus gorbuscha*, subfamily Salmoninae) was determined in an effort to root the coregonine tree with a representative of another subfamily of the Salmonidae. Forty-six variable sites were found, all silent substitutions at third positions of codons, representing 57.5% of the 80 total third position sites. The mean uncorrected pairwise distance to members of the ingroup was 14.9%. Extensive homoplasy was inferred to have occurred along the lineage leading to the ingroup, and within the ingroup itself. This resulted in an inability to resolve relationships within the Coregoninae. Therefore, rooting the coregonine tree with an outgroup that will resolve relationships within the Coregoninae will be contingent upon identifying taxa that have diverged

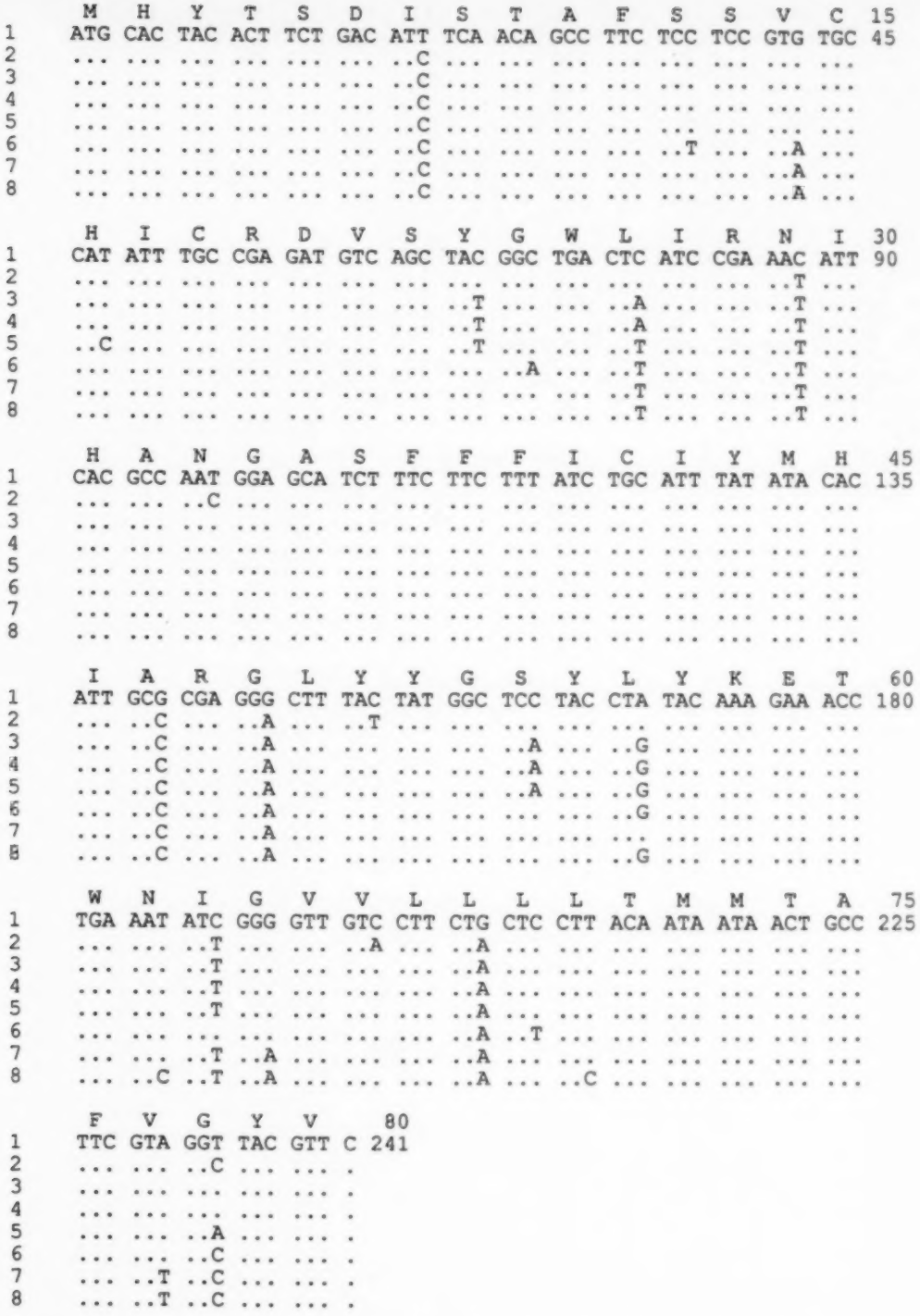
M H Y T S D I S T A F S S V C 15
1 ATG CAC TAC ACT TCT GAC ATT TCA ACA GCC TTC TCC TCC GTG TGC 45
2 ... ...C ... ...
3 ... ...C ... ...
4 ... ...C ... ...
5 ... ...C ... ...
6 ... ...C ... ...T ... ..A ...
7 ... ...C ... ...A ...
8 ... ...C ... ...A ...
H I C R D V S Y G W L I R N I 30
1 CAT ATT TGC CGA GAT GTC AGC TAC GGC TGA CTC ATC CGA AAC ATT 90
2 ... ...T ...
3 ... ...T ... .A ... .T ...
4 ... ...T ... .A ... .T ...
5 ..C ... ...T ... .T ... .T ...
6 ... ...A ... ..T ... .T ...
7 ... ...T ... .T ...
8 ... ...T ... .T ...
H A N G A S F F F I C I Y M H 45
1 CAC GCC AAT GGA GCA TCT TTC TTC TTT ATC TGC ATT TAT ATA CAC 135
2 ... .C ... ...
3 ... ...
4 ... ...
5 ... ...
6 ... ...
7 ... ...
8 ... ...
I A R G L Y Y G S Y L Y K E T 60
1 ATT GCG CGA GGG CTT TAC TAT GGC TCC TAC CTA TAC AAA GAA ACC 180
2 ... ..C ... ..A ... ..T ... ...
3 ... ..C ... ..A ... ...A ... ..G ... ...
4 ... ..C ... ..A ... ...A ... ..G ... ...
5 ... ..C ... ..A ... ...A ... ..G ... ...
6 ... ..C ... ..A ... ...G ... ...
7 ... ..C ... ..A ... ...
8 ... ..C ... ..A ... ...G ... ...
W N I G V V L L L L T M M T A 75
1 TGA AAT ATC GGG GTT GTC CTT CTG CTC CTT ACA ATA ATA ACT GCC 225
2 ... .T ... .A ... ..A ... ...
3 ... .T ... ...A ... ...
4 ... .T ... ...A ... ...
5 ... .T ... ...A ... ...
6 ... ...A ..T ... ...
7 ... .T ..A ... ...A ... ...
8 ... ..C ..T ..A ... ...A ... ..C ... ...
F V G Y V 80
1 TTC GTA GGT TAC GTT C 241
2 ... .C ... .
3 ... ...
4 ... ...
5 ... .A ... .
6 ... .C ... .
7 ... ..T ..C ... .
8 ... ..T ..C ... .

FIG. 1. Sequence of a 241-bp region of the mitochondrial cytochrome *b* gene from eight coregonine species. The nucleotide and inferred amino acid sequences of *S. leucichthys* (sequence 1) are given. Nucleotides identical to the homologous position in the inconnu sequence are denoted by dots. Other sequence identities are as follows: 2, *C. sardinella*; 3, *C. autumnalis*; 4, *C. artedi*; 5, *C. laurettae*, 6, *C. nasus*; 7, *C. pidschian*; 8, *P. cylindraceum*.

TABLE 3. Matrix of percent sequence divergence (below diagonal) among mitochondrial haplotypes for the eight coregonine species analyzed in this study.

		1	2	3	4	5	6	7	8
1	*S. leucichthys*	—							
2	*C. sardinella*	4.1	—						
3	*C. autumnalis*	4.1	3.3	—					
4	*C. artedi*	4.1	3.3	0.0	—				
5	*C. laurettae*	5.0	3.7	1.3	1.3	—			
6	*C. nasus*	5.0	4.1	3.7	3.7	3.7	—		
7	*C. pidschian*	4.7	2.9	3.3	3.3	3.3	2.9	—	
8	*P. cylindraceum*	5.8	4.1	3.7	3.7	3.7	3.3	1.3	—

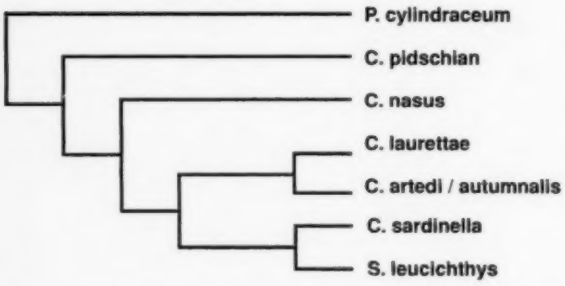

FIG. 2. Parsimony tree built with PAUP relating the variability in the cytochrome *b* gene among all taxa with the fewest events. The cladogram was rooted with the primitive coregonine *P. cylindraceum*. The tree was 28 steps in length, with a CI of 0.89 and an HI of 0.11.

significantly less than members of the salmonine genus *Oncorhynchus*. Selective constraints upon first and second positions of codons in protein-coding genes of salmonids limit the number of informative sites that are available for phylogenetic analysis, as third positions of codons may saturate quickly (Thomas and Beckenbach 1989).

Discussion

Prior to consulting a phylogeny inferred from mtDNA analysis, a review of the history of coregonine taxonomy is appropriate. Koelz (1929) recognized three genera in the subfamily Coregoninae: *Coregonus* (ciscoes and whitefishes), *Prosopium* (round whitefishes), and *Stenodus* (inconnu), based upon fishes collected in the Great Lakes. Berg's (1940) classification of coregonine fishes recognized only two genera, *Coregonus* and *Stenodus*, with the former containing three subgenera, *Coregonus, Leucichthys,* and *Prosopium*. Berg (1948) combined *Prosopium* with *Coregonus* and recognized the ciscoes as a distinct genus, *Leucichthys*.

Norden (1961), in his study of the osteology of the Salmonidae, cited the single nasal flap, retention of parr marks, and the presence of a basibranchial plate as evidence for the more primitive nature of *Prosopium*. Both *Coregonus* and *Stenodus* are marked by the loss of this plate. Norden (1961) reestablished *Prosopium* as a separate genus and placed the ciscoes and whitefishes together in the genus *Coregonus*. Behnke (1972) agreed with Norden (1961) and pointed out that the retention of primitive salmonine characters by *Prosopium* and the lack of maxillary teeth in *Stenodus* argued for the former as the most primitive genus. This was the most recently accepted view of the subfamily (Nelson 1984). Smith and Todd (1993) reviewed previous taxonomic work and identified new characters used in a cladistic analysis that suggested that the relationships in Coregoninae were best summarized as two genera, *Prosopium* and *Coregonus*, with *Prosopium* being the most primitive member of the subfamily. Smith and Todd (1993) included *Stenodus* as a subgenus within *Coregonus*.

Biochemical studies have provided independent tests of coregonine relationships. Ferguson et al. (1978) examined the relationships among European coregonines using protein electrophoresis. Based on this analysis, four groups were established: *C. autumnalis* and *C. a. pollan, C. peled, C. albula,* and a group called "*C. lavaretus* species." These groups corresponded to the divisions of whitefishes and ciscoes established by Shaposhnikova (1970), who used osteological characters to separate the European species. Bodaly et al. (1991) examined gene frequencies in nine species of Old and New World coregonines and found six distinct genetic groups based on genetic distance calculations. *Stenodus leucichthys* was the most distantly related taxon examined. A second closely related group consisted of *C. autumnalis, C. a. pollan,* and *C. artedi*. The *C. pidschian* complex (lake whitefishes) formed a third group, with differentiation on the level of geographic races occurring between the Bering and Mississippian forms, which presumably arose during the Wisconsin glaciation. The last three groups consisted of *C. nasus, C. sardinella*, and *C. albula* (vendace), and *C. peled* (peled). Neither study included a member of the primitive genus *Prosopium* as an outgroup.

The cladistic analysis of the mtDNA sequence data provides support for recent morphological and genetic classifications of coregonines. The two members of the subgenus *Coregonus* (*C. nasus* and *C. pidschian*) emerged as distinct sister taxa, corroborating previous morphological and genetic assays (Bodaly et al. 1991; Smith and Todd 1993). The members of the subgenus *Leucichthys* (ciscoes) separated into two groups. *Coregonus artedi, C. autumnalis,* and *C. laurettae* formed one assemblage. *Coregonus sardinella* comprised the second assemblage. *Coregonus autumnalis* and *C. artedi* shared identical sequences for this portion of the cytochrome *b* gene, suggesting that the splitting of this lineage is rather recent. Bodaly et al. (1991) suggested that *C. autumnalis/artedi* were probably geographic races of the same wide-ranging species, and the mtDNA data provide strong support for this conclusion.

The position of *Stenodus* as a derived member of the subfamily relative to the primitive outgroup *Prosopium* is corroborated (Smith and Todd 1993). Smith (1957) considered *Stenodus*, with its resemblance to the subfamily Salmoninae, its less reduced

teeth, and its tendency to undertake extensive migrations, as probably closest to the ancestral coregonine. The mtDNA sequence data provide no support for such a conclusion.

The position of *C. sardinella* is also derived, relative to *P. cylindraceum*. The position of the former calls into question the monophyletic nature of the subgenus *Leucichthys*. This question needs further study.

Our mtDNA results shed light on recent investigations of the colonization of the Mackenzie River by ciscoes of unknown origin, following the retreat of the Wisconsin glacier. Documented spawning runs of *C. autumnalis* in North America occur within the Mackenzie River system, in five west bank tributaries (Peel, Arctic Red, Mountain, Carcajou, and Liard rivers) and one east bank tributary (Great Bear River). Both the Liard and Peel rivers were hypothesized to have been tributary to the Yukon River following Wisconsin glaciation (Lindsey and McPhail 1986). Bickham et al. (1989) suggested that the colonization of the Mackenzie River following the Wisconsin glaciation may have proceeded bidirectionally. One possible source of the Mackenzie ciscoes was from a stream capture of ancestral *C. laurettae* from the headwaters of the Yukon River into the Liard River (Lindsey and McPhail 1986). Alternatively, *C. laurettae* may have dispersed farther east along the Beaufort Sea coast than previously believed, entered the Mackenzie River from its delta region, and dispersed south (Bickham et al. 1989). Neither hypothesis has been tested genetically.

The identical sequences found among *C. autumnalis* from the Liard and Peel rivers and *C. artedi* from the Laurentian Great Lakes suggest that the colonization of the Mackenzie may have proceeded from the east, from *C. artedi* of Mississippian refugia (Dillinger 1989). The colonization corridor may have been a postglacial connection between Great Bear Lake and inland lakes of Mississippian refugia; this would explain the presence of *C. artedi* in Great Bear Lake. Sampling of ciscoes from Great Bear Lake is clearly indicated. The relatively substantial sequence divergence between Yukon River *C. laurettae* and *C. autumnalis* (1.3%) and the absence of sequence divergence between the Great Lakes *C. artedi* and Mackenzie River ciscoes appear to rule out the Yukon River population as the ancestral stock of Mackenzie River ciscoes.

Pairwise sequence divergence estimates among the eight taxa surveyed ranged from 0.0 to 5.8% (Table 3). These results suggest that the origin of putative coregonine taxa spans the relatively recent Pleistocene glaciations (*C. autumnalis/artedi*) to nearly 6 million yr B.P. for the divergence of *P. cylindraceum* and *S. leucichthys*. This range of values was calculated assuming that the rate of mtDNA sequence divergence in coregonines lies between that in mammals (2% per million yr per pair of lineages; Brown et al. 1982) and that in sharks (1% per million yr per pair of lineages) (A. Martin, personal communication), groups for which molecular clocks have been calibrated. Directed estimates of genetic distance must be used with caution owing to the biased rate of substitution along the cytochrome *b* gene and the relatively short portion of DNA analyzed (Kocher et al. 1989; Martin et al. 1990; Irwin et al. 1991). The greatest pairwise distance found in this study was between *P. cylindraceum* and *S. leucichthys* (5.8%), nearly identical to that between the congeners *Coregonus huntsmani* and *C. artedi* (5.82%), estimated by restriction fragment length polymorphism (RFLP) analysis of mtDNA (Bernatchez et al. 1991). These results substantiate the conclusion of Smith and Todd (1993) that members of the genus *Coregonus* including whitefishes, ciscoes, and inconnu are a diverse assemblage that does not form a monophyletic group within the Coregoninae.

An analysis of tree topologies produced by both neighbour joining (NJT) and UPGMA methods (trees not shown) maintains the relationships between *C. autumnalis*, *C. artedi*, and *C. laurettae*, and *C. pidschian* and *P. cylindraceum*. Relationships affected by using these distance trees include those between *S. leucichthys* and *C. sardinella*, and *C. nasus* with *C. pidschian* and *P. cylindraceum*. The use of UPGMA clustering must be regarded with caution, if the assumption of constant rate of evolutionary change has been violated. The NJT methodology does not suffer from this same problem. The tree produced by this method (with *P. cylindraceum* as an outgroup) is similar to the one in our study. NJT will produce a different tree when *S. leucichthys* is the outgroup, but as mentioned above, there appears to be no justification for that approach. We feel that the parsimony method used is an acceptable and appropriate methodology for phylogenetic analysis.

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Changes in Habitat Use and Feeding Chronology of Juvenile Rainbow Trout (*Oncorhynchus mykiss*) in Fall and the Onset of Winter in Silver Creek, Idaho

Michael D. Riehle and J.S. Griffith[1]

Department of Biological Sciences, Idaho State University, Pocatello, ID 83209, USA

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We assessed changes in density, distribution, and microhabitat of age-0 rainbow trout (*Oncorhynchus mykiss*) in Silver Creek, a partially spring-fed stream, by periodic snorkeling in August 1987 through January 1988. We examined trout stomach contents and invertebrate drift samples in diel collections in August, September, October, and January to test if the period of feeding shifted from daytime to nighttime, concurrent with a transition to day concealment. In late September, fish aggregated briefly during the day and then began to conceal themselves in macrophyte beds, undercut banks, and submerged sedges and grasses along streambanks as temperature dropped below 8°C in early October. Fish emerged from concealment at night, and numbers of trout visible were greatest 30–60 min after sunset and about 30 min before sunrise. Periods of peak feeding changed from afternoon and evening in August and September, when fish were day active, to mainly at night in October after the initiation of day concealment. Trout did not feed upon abundant chironomids in the daytime drift in October. In January, fish fed at 1–4°C on mayflies, and stomachs were fullest in the early morning. Observations suggest that Silver Creek trout experienced a metabolic deficit that began in September.

Nous avons évalué les changements dans la densité, la répartition et le micro-habitat de la truite arc-en-ciel (*Oncorhynchus mykiss*) d'âge 0 dans le ruisseau Silver, cours d'eau partiellement alimenté par source, en effectuant, entre août 1987 et janvier 1988, des examens périodiques en plongée libre. Nous avons examiné le contenu de l'estomac des truites et des échantillons d'invertébrés présents dans le courant en les prélevant sur un nycthémère en août, septembre, octobre et janvier afin de vérifier si la période d'alimentation passait du jour à la nuit en même temps que les poissons commençaient à se cacher dans la journée. À la fin septembre, les poissons se rassemblaient brièvement pendant la journée puis commençaient à se cacher dans les bancs de macrophytes, à l'abri des sous-berges et parmi les carex et les herbes immergées, le long des rives, quand la température est passée au-dessous de 8°C au début d'octobre. Les poissons sortaient de leur abri la nuit, et le nombre de truites visibles était au maximum 30 à 60 min après le coucher du soleil et environ 30 min avant le lever du soleil. Les périodes où l'alimentation était au maximum, c'est-à-dire l'après-midi et le soir en août et septembre, quand les poissons étaient actifs le jour, se situaient principalement la nuit en octobre après que les poissons avaient commencé à se cacher le jour. Les truites ne se nourrissaient pas des chironomidés qui abondaient dans le courant pendant le jour en octobre. En janvier, elles se nourrissaient d'éphémères à 1–4°C, et les estomacs étaient pleins au maximum tôt le matin. Les observations semblent indiquer que les truites du ruisseau Silver connaissaient un déficit métabolique qui commençait en septembre.

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Winter is a stressful period for stream-dwelling salmonids. Low water temperature slows the rate of digestion and may limit the amount of energy available for metabolism and growth, even if food is available and feeding occurs (Cunjak and Power 1987). A reduction in water temperature reduces the swimming performance of trout (Hartman 1963), which impairs their ability to escape endothermic predators.

At the onset of winter, juvenile salmonids may form aggregations in open water, especially in thermal refuges (Cunjak and Power 1986), or may conceal themselves in woody debris, in interstices of the substrate (Hartman 1965), or under undercut banks (Hillman et al. 1987). Juvenile rainbow trout (*Oncorhynchus mykiss*) that were concealed during the day have been observed to emerge from concealment cover at night (Campbell and Neuner 1985; Contor 1989). Reasons for such a shift in habitat and abandonment of the presumably secure cover of the substrate remain unclear. One hypothesis is that juvenile rainbow trout move out of the substrate at night to feed when they are not vulnerable to day-active predators.

Feeding activity during winter has been described for stream-dwelling juvenile rainbow trout (Maciolek and Needham 1951; Needham and Jones 1956; Cunjak and Power 1987), Atlantic salmon (*Salmo salar*) (Thomas 1962; Smirnov et al. 1976; Riddell and Leggett 1981; Cunjak 1988a; but see Metcalfe and Thorpe 1992), brown trout (*Salmo trutta*) (Maciolek and

[1]Author to whom correspondence should be addressed.

Needham 1951; Reimers 1957; Thomas 1962; Mortensen 1985; Cunjak and Power 1987), and brook trout (*Salvelinus fontinalis*) (Cunjak and Power 1987). However, these researchers did not observe whether feeding occurred at night or during the day.

This study had two main objectives. The first was to assess abundance, distribution and microhabitat use by age-0 rainbow trout in Silver Creek at the onset of winter. We were especially interested in whether fish concealed themselves in the dense beds of perennial aquatic macrophytes that were the main source of cover in the study area. The second objective was to assess the extent of feeding by age-0 rainbow trout and its diel chronology in Silver Creek. We tested if the period of feeding shifted from daytime to nighttime, concurrent with a transition to diurnal concealment behavior in winter.

Study Area

Silver Creek is a spring-influenced tributary of the Little Wood River located on the northern edge of the Snake River Plain in Blaine County, south-central Idaho. The 400-m-long study area, chosen for its abundance of juvenile trout and its accessibility, began approximately 1.5 km downstream from the confluence of Grove and Stalker creeks. The study area was a low-gradient (0.17%), meandering reach that ranged from 25 to 30 m wide. Discharge was relatively constant at about 3 $m^3 \cdot s^{-1}$ during the study period of August 1987 through January 1988, with a slight increase in late summer and early autumn. Specific conductance in August was 365 $\mu S \cdot cm^{-1}$. Water temperature, monitored with a Peabody Ryan recording thermograph in the study area, peaked at 17°C in August. Because of the spring-influenced tributaries, midwinter temperature remained near 4–5°C except for 2 d in early January when daily minima reached 0°C. No anchor ice was observed in the study area, and the only surface ice occurred along stream margins in January.

Although no comprehensive determination of habitat availability was regularly conducted during the study period, we surveyed habitat across 10 transects equally spaced along the study area during the last week of August. Water depth averaged 67 cm (range 61–73 cm). Dense beds of the macrophytes *Chara vularis* and *Potamogeton pectinatus* covered an average of 66% (range 55–74%) of the stream bottom from bank to bank. Substrate under the macrophytes ranged from medium silt to medium sand (0.016–0.5 mm in diameter, based on Platts et al. 1983) and averaged 14 cm deep. Channels (seven at each transect) through the macrophyte beds had fine gravel substrate, 4–8 mm in diameter. No cobble or boulder substrate was present. Undercut or vertical banks extended along >90% of the study area, and there were few margins shallower than about 25 cm. Riparian vegetation consisted of yellow willow (*Salix lutea*), American bulrush (*Scirpus americanus*), sedge (*Carex*) sp., and grasses. By the end of the study period in January, the stream surface area covered by macrophytes had gradually declined by approximately 30%. Water surface elevation, measured on a staff gage in the study area, increased a few centimetres in September, remained nearly constant through November, and dropped in January to a level 12 cm below that in August.

Rainbow trout were the most abundant salmonid in the study area. A few juvenile brown and brook trout were also present, but were not included in the study. Other fish species in the study area in late August, in decreasing order of abundance, were mountain whitefish (*Prosopium williamsoni*), bridgelip sucker (*Catostomus columbianus*), longnose dace (*Rhinichthys cataractae*), and Wood River sculpin (*Cottus leiopomus*). In September and October, most adult trout and nonsalmonids moved out of the study area to deeper water downstream. During winter, a few juvenile mountain whitefish were observed in the study area. Predators that remained in the study area during fall and winter included mink (*Mustela vison*), great blue herons (*Ardea herodias*), common mergansers (*Mergus merganser*), and belted kingfishers (*Ceryle alcyon*).

Methods

We divided the study area into three sites, two each of approximately 100 m at either end and a central site of 200 m. We snorkeled to count fish and assess microhabitat use in each of the distal sites and captured trout by electrofishing for diet analysis in the central site.

Trout Abundance and Distribution

We enumerated juvenile rainbow trout along the stream margin and in midstream by snorkeling along four parallel lanes in each site. The lanes, each 2 m wide and spaced equally from each other, were located along the west stream margin, west midchannel, east midchannel, and east stream margin. A snorkeler crawled slowly upstream and counted fish. At colder temperatures, trout are more easily disturbed (Bustard and Narver 1975). Sudden movements or splashing were avoided to prevent fish from swimming ahead of the snorkeler. At night, we followed the same procedure, using a dive light with a red filter. Daytime snorkeling was conducted between 10:00 and 17:00 Mountain Daylight Time (MDT; MST in November and January), and nighttime snorkeling was initiated 2 h after sunset. Water clarity, which we defined as the distance at which a juvenile trout could be clearly seen by the snorkeler, was 2.5–3.5 m during day and night throughout the study period. Snorkeling was completed at least monthly from August through November 1987 and in January 1988 (Table 1).

To assess the relationship between light intensity and emergence of fish from concealment cover, we monitored trout density at 20-min intervals during dusk and dawn of the September and November sample dates. A snorkeler counted fish along a 2-m-wide, 50-m-long lane along the bank in the central study site. We used a Li-Cor model LI-200SA pyranometer to measure minimum and maximum irradiance during each interval.

Microhabitat Use

Night microhabitat use by juvenile rainbow trout was assessed at five periods from summer through winter (8–9 August, 25–27 September, 16 October, and 13 November 1987 and 20 January 1988). Day habitat use was examined only in August and September; thereafter, most juvenile rainbow trout were concealed during the daytime. We assessed microhabitat use along the four lanes used to estimate fish density at the same time habitat use was assessed. A snorkeler moved upstream and marked fish positions with a flag at the focal point of each fish, taking care to avoid disturbing other trout.

During night dives, we noted the position of each fish before it reacted to the dive light. Once the light beam struck a fish, its typical response was to remain in the same position and then slowly sink to the substrate. As the snorkeler approached more closely, the fish either slowly moved away or swam up in the water column in an erratic manner and then darted into cover

TABLE 1. Densities (fish · m^{-2}) of juvenile rainbow trout estimated by snorkeling 2-m-wide lanes along stream margins and in midchannel portions of Silver Creek, Idaho, in 1987 and 1988.

	Temperature at time of snorkeling	Upper site		Lower site	
		Stream margin	Midchannel	Stream margin	Midchannel
Day					
3 August	17.0	11.9	26.7	7.4	34.7
8 August	16.0	16.7	30.0	18.5	48.0
15 August	15.0	25.0	49.8	12.2	54.0
6–7 September	13.0	5.7	59.0	14.0	61.0
21 September	11.0	2.0	41.0	4.8	38.6
25 September	13.0	5.7	39.4	4.8	48.0
3 October[a]	8.5	0	0	0	0
3 October[b]	13.0	0.4	0	1.4	1.1
16 October	8.0	0.4	0	0.6	0
Night					
17 August	14.0	56.1	20.2	32.4	34.9
26 September	11.0	40.6	20.6	31.5	23.3
17 October	9.5	3.0	1.9	15.8	2.0
13 November	8.0	10.4	2.3	4.5	0.6
20 November	6.0	6.1	0.2	2.0	0.6
20 January	2.0	4.6	0	3.0	0

[a]Morning count conducted at 10:00 MDT.
[b]Afternoon count conducted at 16:30.

within a metre of its original location. Because individual trout were usually visually isolated from one another by macrophytes, a disturbed fish seldom affected others. The snorkeler trained during nights with a full moon when fish could be observed without the aid of a dive light and then when the light was turned on. Awareness of this range of behavior aided the snorkeler in detecting when a fish had been disturbed, so that it could be disregarded. All data for night microhabitat analysis were collected at new moon or when the moon was less than one quarter full.

Microhabitat variables were measured immediately for those focal points marked during the day and the following morning for those marked at night. Variables measured were water depth, focal point elevation (distance above the substrate), focal point water velocity, adjacent velocity (maximum velocity within 30 cm of the focal point), substrate type, type of cover closest to the focal point, and distance to cover. Water velocity was measured with a Marsh–McBirney model 201 velocity meter (accuracy ±2%) with a 3.5-cm-diameter measuring probe. Cover was defined as the nearest structure within 2 m that we felt could provide concealment for juvenile rainbow trout. An "other" cover category was used to include macrophytes other than *Chara* or *Potamogeton* and woody debris. Substrate below the focal point of a fish was categorized as gravel, sand, silt, or clay according to the particle size classification of Platts et al. (1983). Gravel < 10 cm in diameter was the largest inorganic substrate and was too small to provide concealment cover for juvenile rainbow trout.

Based on habitat similarities and failure to detect significant differences (alpha = 0.05, used throughout the paper) in microhabitat values between the two sites, microhabitat data were pooled. Differences in night microhabitat utilization from summer through winter were assessed using a Kruskal–Wallis test. A Wilcoxon rank-sum test was performed on the day microhabitat utilization data because only two sets of observations were made before trout adopted concealment behavior. Changes in the type of cover and substrate used before and after the initiation of daytime concealment were evaluated using a chi-square test.

Feeding Chronology

We assessed the diet of age-0 rainbow trout during 24-h periods in late summer (5–6 August 1987), early fall (19–20 September 1987), early winter (24–25 October 1987), and midwinter (23–24 January 1988). The first three collections encompassed 24 periods beginning at 09:00 and ending at 06:00. Stomach contents were collected from 15 fish at 3-h intervals using gastric lavage. In January, diet was assessed at 09:00, 15:00, and 09:00 because too few fish were available to enable more frequent collection. Nocturnal illumination was provided by a first quarter moon on the August date and conditions intermediate between one quarter and new moon on the other dates.

Age-0 rainbow trout were captured with a backpack electrofisher and anesthetized with tricane methanesulfonate. Gastric lavage was employed, using a number 18 hypodermic needle and a 30-mL syringe to flush the cardiac portion of the stomach. Samples were fixed immediately in 10% formalin. We measured total length of trout to the nearest millimetre and released them after marking with a partial fin clip to preclude their reuse on that sample date.

In the laboratory, invertebrates from trout stomachs were identified under a dissecting microscope to the lowest feasible taxonomic level (usually genus) and weighed to the nearest milligram. Stomach content wet weights were divided by live body weight and multiplied by 100 to yield a relative stomach content wet weight (RWW) for each fish as done by Angradi and Griffith (1989). We used $\log_e(x + 1)$ transformation to normalize the RWW data and single-factor analysis of variance to test for

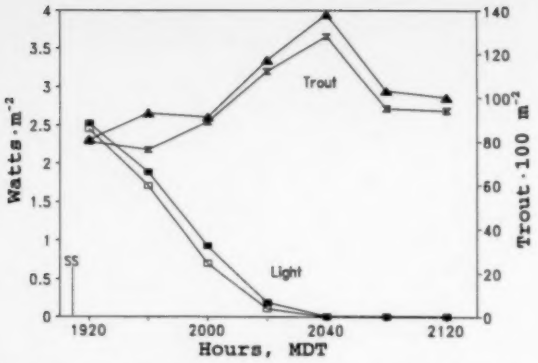

FIG. 1. Changes in density of age-0 rainbow trout visible to snorkelers along the stream margin and light intensity at dusk in Silver Creek, Idaho, on 5 September (open symbols) and 6 September (solid symbols) 1987.

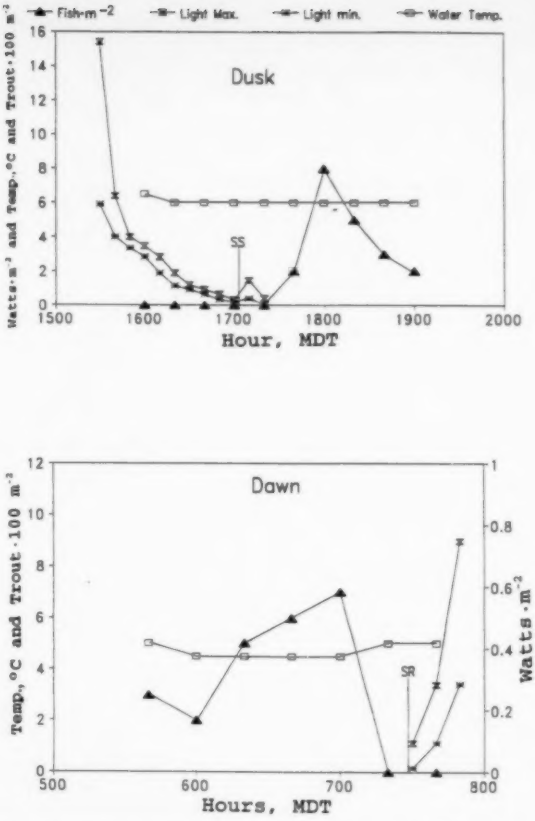

FIG. 2. Changes in density of age-0 rainbow trout visible to snorkelers along the stream margin, maximum and minimum light intensity during each 20-min observation period, and water temperature in Silver Creek at dusk on 20 November and at dawn on 21 November 1987. SS, sunset; SR, sunrise.

diel changes in RWW. Tukey's multiple-comparison test was used to identify the occurrence of peak feeding.

We collected surface and subsurface invertebrate drift at the upstream end of the site from which trout were captured for diet analysis. Six drift nets with 10 × 13 cm openings and 0.24-mm mesh were set for 20 min during each period when trout were collected for diet analysis. Three of the nets were set so that the water surface was at the middle of the net mouth and 5 cm of the net opening was under water, and three nets were set at 0.6 the depth of the water. Following Allan and Russek (1985), flow through each net was estimated from triplicate readings of water velocity within the net mouth at the beginning and end of each collection period. In the laboratory, invertebrates were identified under a dissecting microscope to the lowest feasible taxonomic level, usually genus, and drift was expressed as number of organisms per cubic metre of water filtered. Mean coefficient of variation for each diel sample ranged from 0.31 to 0.39.

Results

Trout Abundance and Distribution

The density of age-0 rainbow trout in daytime peaked in early September and decreased gradually through mid-September in both sites (Table 1). Peak daytime densities were observed on 6–7 September (59.0 and 61.1 fish·100 m^{-2} for upper and lower sites, respectively) in the midchannel snorkel lanes. In August and September, the density of trout remained highest in the midchannel lanes in the day, with densities over 35 fish·100 m^{-2}. However, fish were more abundant along the stream margins than in midchannel at night (chi-square, $P < 0.0001$ for both sites). Overall night densities in August and September were similar to the daytime estimates.

We last observed day-active juvenile trout in the study area on 25 September at 13°C. On that date, all age-0 rainbow trout seen were in aggregations, from a few dozen up to 200 individuals, that included a few age-0 whitefish and age-0 brown trout. Between 25 September and 3 October 1987, water temperature began to drop below 8°C at night and trout began to conceal themselves from the snorkeler during daylight hours. During the morning of 3 October at 8°C, we observed no fish; at 16:30, water had warmed to13°C and we observed 1.4 fish·100 m^{-2} (Table 1). A few individual trout (<0.6 fish·100 m^{-2}) were visible in open water during the day on 16 October at 8°C.

After fish began to conceal themselves during the day, nighttime densities gradually declined from a peak of 15.8 fish·100 m^{-2} on 17 October to much lower levels in November and January (Table 1). A greater proportion of the population was noted along stream margins than in midchannel (chi-square, $P < 0.05$ for the upper site and $P < 0.01$ for the lower site).

Nocturnal Emergence From Concealment

In September, the number of age-0 rainbow trout observed along the stream margins in the central study site increased as light decreased at dusk (Fig. 1). On 5 September, density was approximately 80 fish·100 m^{-2} when light levels began to decline. When the light meter indicated the lowest measurable level (0.001 W·m^{-2}) 40 min after sunset, density was 127 fish·100 m^{-2} in the two subsequent census periods. On 6 September, we observed the same pattern, with a maximum of 138 fish·100 m^{-2} 30 min after sunset (Fig. 1).

On 20–21 November, a similar change in trout density at dusk occurred, except that no unconcealed fish were evident in full daylight (Fig. 2). Trout left concealment cover and became visible to the snorkeler just as the amount of light became too low to measure with our equipment. After peaking 1 h after

TABLE 2. Summary statistics for microhabitat variables of juvenile rainbow trout in Silver Creek, Idaho, in 1987 and 1988. Fish were concealed during the day during October–January.

Variable	Month and *N*	Day		Night	
		Mean	SD	Mean	SD
Total depth (cm)	August,	45	12	45	11
Focal elevation (cm)	*N* = 87 (day), 8	3	7	4	
Adjacent velocity (cm)	*N* = 53 (night)	23	7	29	10
Focal velocity (cm)		15	7	13	8
Distance to cover (cm)		28	23	24	18
Total depth (cm)	September,	51	9	43	12
Focal elevation (cm)	*N* = 49 (day), 8	3	7	3	
Adjacent velocity (cm)	*N* = 98 (night)	29	10	25	9
Focal velocity (cm)		14	8	11	7
Distance to cover (cm)		48	30	38	29
Total depth (cm)	October,			37	14
Focal elevation (cm)	*N* = 48 (night)			37	2
Adjacent velocity (cm)				22	8
Focal velocity (cm)				48	3
Distance to cover (cm)				21	19
Total depth (cm)	November,			35	12
Focal elevation (cm)	*N* = 44			5	5
Adjacent velocity (cm)				16	9
Focal velocity (cm)				4	2
Distance to cover (cm)				14	10
Total depth (cm)	January,			27	9
Focal elevation (cm)	*N* = 22			1	1
Adjacent velocity (cm)				16	9
Focal velocity (cm)				3	1
Distance to cover (cm)				13	9

sunset, densities observed at 19:00 (2–5 fish·100 m^{-2}) were similar to those we had noted on 20 November along the margins of the upper and lower sites 2 h after sunset (2.0–6.1 fish·100 m^{-2}) (Table 1).

The pattern for the dawn period in November (Fig. 2) was the reverse of dusk. Before dawn, trout densities rose from 2–3 to 7 fish·100 m^{-2} about 30 min before sunrise. When the light meter indicated the lowest measurable level about 10 min prior to sunrise, all trout had concealed themselves in cover.

Microhabitat Use

Daytime microhabitat used by age-0 rainbow trout in the study sites changed from August to September (Table 2). Fish used significantly deeper water (mean = 51 cm) in September when they were in aggregations than in August (mean = 45 cm). Although focal velocities remained similar from August to September, adjacent water velocity increased significantly from a mean of 23 cm·s^{-1} in August to 28 cm·s^{-1} in September. Fish were found at a significantly greater distance from cover in September (mean = 28 cm) than in August (mean = 48 cm). Daytime focal elevations did not change significantly. In August, 44% of the fish were observed over *Chara*; this changed to 31% in September, with 57% over gravel, a significant change. *Chara* was the closest cover for a greater proportion of fish (92%) in September than in August (68%), also a significant change.

Nighttime microhabitat changed as temperature declined (Table 2). Juvenile rainbow trout progressively moved into shallower water. Mean depth used by fish decreased significantly from 45 cm in August to 27 cm in January. Focal elevation that ranged from a mean of 4–7 cm·s^{-1} in August–November fell to <1 cm·s^{-1} in January, a significant change. Juvenile rainbow trout gradually adopted positions in significantly slower water that were significantly closer to cover. Their mean focal velocity fell from 13cm·s^{-1} in August to 3 cm·s^{-1} in January, and distance to cover decreased from a mean of 24 to 13 cm in January.

Before the shift to day concealment, 54% of the juvenile rainbow trout selected nighttime stations over *Chara*. After the fish began to conceal themselves during the day, they were associated with silt and clay substrates at night significantly more frequently (55%) than with *Chara* (29%). Before adopting daytime concealment behavior, the closest cover at night was *Chara* for 74% of the fish. After the shift to day concealment, trout were observed significantly more frequently near the stream margin and flooded grasses at night (40%) than before, with 53% of the trout closer to *Chara* than to other available cover.

Feeding Chronology

The mean length of age-0 trout used for the diet analysis increased from 68.4 mm (range 49–92 mm) in early August to 85.5 mm (range 58–99 mm) in mid-September, 81.7 mm (range 63–100 mm) in late October, and 91.2 mm (range 68–122 mm) in late January.

During the 5–6 August sample of drift and diet, mean RWW

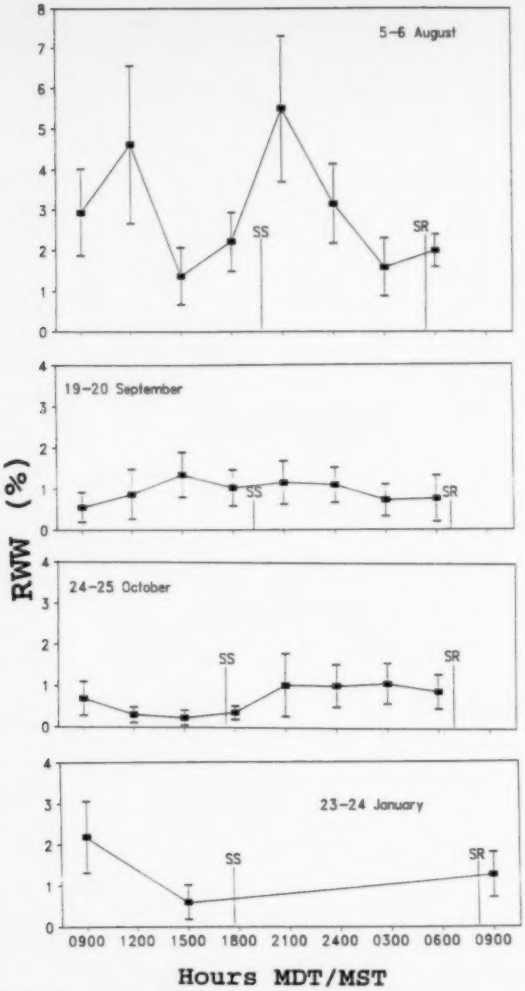

FIG. 3. Diel change in mean relative wet weight of stomach contents (RRW) of age-0 rainbow trout in Silver Creek, August 1987–January 1988. Vertical bars indicate 95% confidence limits. SS, sunset; SR, sunrise.

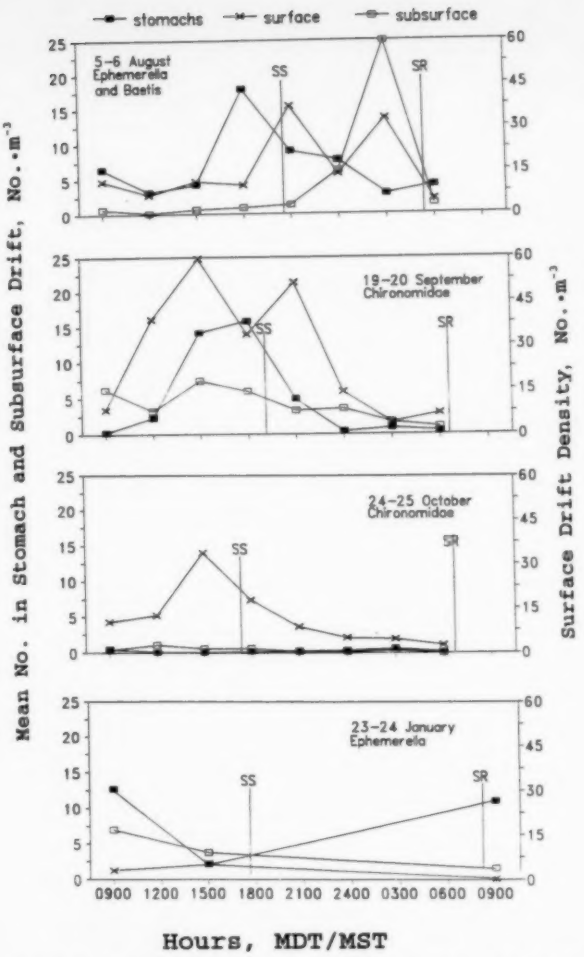

FIG. 4. Diel changes in mean number of prey items eaten by age-0 rainbow trout and surface and subsurface drift of those taxa in Silver Creek, August 1987–January 1988. SS, sunset; SR, sunrise.

was 3.8% for the diel period (Fig. 3) and the mean number of food items per stomach was 11.4. RWW peaked at 5.5% in the 21:00 sample just after sunset and at 4.6% at 12:00 (ANOVA, $P < 0.01$ and Tukey test, $P < 0.05$). Diel change in weight of food in the stomachs was correlated with the density of surface drift ($r^2 = 0.59$, $P < 0.05$), but not with water temperature that ranged from 13.0 to 17.4°C or the density of subsurface drift. Mayflies comprised 78% by number of the food ingested during the diel period. Densities of the relatively large mayfly *Ephemerella inermis* and the smaller *Baetis* sp. increased sharply in the surface drift from 18:00 to 21:00. Mayflies were eaten then, but their frequency in stomachs declined at night (Fig. 4; Table 3). Chironomids were the next most utilized abundant food item after mayflies. They were eaten during the day and at dusk, but seldom at 24:00 or 03:00 despite their continued abundance in the surface drift. Mean density (per cubic metre of water filtered) of invertebrates collected in drift nets (surface and subsurface combined) over the diel period was 35.4 (SD = 13.80) chironomids and 46.9 (SD = 17.35) members of all other taxa combined.

During the 19–20 September sample, mean daily RWW declined to a mean of 1.0% with no significant peaks (Fig. 3), and the mean number of food items per stomach was 9.5. Weight of prey in stomachs was significantly correlated with surface drift ($r^2 = 0.57$, $P < 0.05$) and water temperature that ranged from 9.0 to 12.5°C ($r^2 = 0.65$; $P < 0.05$), but not with subsurface drift. The most numerous prey in the stomachs were larval and pupal Chironomidae, and mayflies comprised only 15% of items ingested (Table 3). During the 15:00 and 18:00 samples, immature Chironomidae comprised 75 and 61% of the stomach contents, respectively. This peak in the number of chironomids in the stomachs in the afternoon followed a corresponding increase in their density in the surface drift (Fig. 4). Mean density of invertebrates collected in drift nets (surface and subsurface combined per cubic metre of water) over the diel period was 30.6 (SD = 12.35) chironomids and 63.9 (SD = 22.37) members of all other taxa combined.

On 24–25 October, mean RWW was 0.8% (Fig. 3), with a mean of 5.1 food items per stomach. Weight of food in the

TABLE 3. Composition of stomach contents of age-0 rainbow trout captured in August, September, and October in Silver Creek, Idaho. For each sample (N = 15 trout), the number of each invertebrate taxon present and its percentage (in parentheses) of that sample are given.

Taxon	Hour of sampling (MDT)							
	09:00	12:00	15:00	18:00	21:00	24:00	03:00	06:00
5–6 August								
Ephemerella inermis	12 (7)	4 (3)	5 (4)	8 (3)	99 (34)	104 (56)	26 (25)	27 (22)
Baetis sp.	85 (51)	43 (32)	59 (52)	261 (83)	36 (12)	12 (8)	17 (23)	34 (28)
Other Ephemeroptera	36 (21)	48 (36)	18 (16)	6 (3)	74 (26)	20 (12)	13 (28)	17 (14)
Chironomidae	13 (8)	14 (10)	9 (8)	15 (5)	66 (22)	9 (6)	5 (7)	8 (7)
Other Diptera	3 (2)	12 (8)	7 (6)	5 (2)	1 (1)	0	1 (1)	0
Other aquatic invertebrates	18 (11)	14 (11)	14 (12)	18 (7)	13 (5)	11 (7)	12 (16)	35 (29)
Terrestrial invertebrates	1 (1)	0	0	0	0	0	0	0
Total number	168	135	112	314	189	156	74	122
19–20 September								
Ephemerella inermis	0	2 (2)	1 (1)	0	0	0	1 (2)	1 (2)
Baetis sp.	7 (14)	46 (41)	15 (6)	68 (18)	12 (7)	7 (9)	3 (7)	7 (17)
Other Ephemeroptera	3 (6)	3 (3)	9 (4)	5 (2)	9 (4)	6 (9)	2 (4)	0
Chironomidae	4 (8)	35 (31)	213 (76)	237 (62)	74 (46)	6 (8)	13 (20)	7 (17)
Other Diptera	4 (8)	1 (1)	2 (1)	13 (3)	2 (1)	4 (6)	2 (4)	11 (27)
Other aquatic invertebrates	32 (64)	20 (18)	28 (10)	58 (16)	49 (31)	19 (29)	22 (51)	14 (33)
Terrestrial invertebrates	0	5 (4)	10 (4)	4 (1)	15 (9)	28 (39)	0	1 (2)
Total number	50	112	278	385	161	72	43	41
24–25 October								
Ephemerella inermis	0	3 (1)	0	0	0	0	0	0
Baetis sp.	6 (12)	2 (6)	2 (13)	6 (21)	71 (48)	50 (34)	19 (22)	16 (18)
Other Ephemeroptera	6 (12)	2 (5)	1 (7)	0	13 (9)	8 (6)	5 (5)	9 (10)
Chironomidae	7 (14)	1 (3)	1 (7)	3 (11)	4 (2)	5 (3)	9 (10)	3 (3)
Other Diptera	1 (2)	1 (3)	3 (20)	1 (4)	22 (15)	5 (3)	10 (11)	6 (6)
Other aquatic invertebrates	30 (59)	30 (80)	8 (54)	18 (65)	34 (23)	78 (53)	45 (50)	58 (63)
Terrestrial invertebrates	1 (2)	0	0	0	1 (1)	2 (1)	2 (1)	1 (1)
Total number	51	37	15	28	145	148	90	93

stomachs was not correlated with temperature (6.0–10.5°C) or with surface or subsurface invertebrate drift, but was nearly negatively correlated with surface drift density (P = 0.06). Density of chironomids in the surface drift during the day in October was similar to that in September, but much reduced from September densities at night. In October, juvenile rainbow trout in the study area did not exploit the daytime food resource of chironomids that in September had provided up to 75% of the diet. Chironomids comprised 5% of items ingested by fish in October (Fig. 4; Table 3). Trout collected throughout the night had eaten *Baetis* mayflies, and a significant peak in feeding occurred during the night at 03:00 (ANOVA, $P < 0.01$ and Tukey test). Mean densities of chironomids and of invertebrates other than chironomids collected over the diel period were 12.2 (SD = 4.02) and 14.9 (SD = 5.21) organisms, respectively, per cubic metre of water filtered by the drift nets.

In the three samples on 24–25 January, water temperature was 1.1–3.9°C. Mean RWW decreased significantly from 2.1% at 09:00 to 0.5% at 15:00 (ANOVA, $P < 0.05$ and Tukey test) (Fig. 3). A mean of 22.8 food items per stomach was found, and the mayfly nymphs *E. inermis* and *Baetis* sp. together comprised 81–85% of that total (Fig. 4; Table 4). Terrestrial insects and adult Ephemeroptera were absent from the drift samples. Mean density of drift in the three samples was 5.4 (SD = 2.05) chironomids and 20.5 (SD = 7.38) members of other taxa (mostly mayflies drifting at night) per cubic metre of water filtered.

Discussion

Water temperatures in Silver Creek during winter were moderated a few degrees in comparison with most trout streams in the region, but were cold enough to induce behavioral changes in trout. Age-0 rainbow trout in the study sites briefly formed large clusters during the day in late September just prior to the shift in day concealment. Cunjak and Power (1986) found brook trout and brown trout in aggregations in headwaters of the Credit River, Ontario, at temperatures between 0.1 and 5.5°C, although they did not find trout subsequently concealed during the day. Unlike in Silver Creek, surface ice in their study area served as cover. Cunjak and Power (1986) found that trout used deeper water when they formed aggregations. Silver Creek trout similarly used deeper water and were farther from cover in

TABLE 4. Composition of stomach contents of age-0 rainbow trout collected on 23–24 January 1987 from Silver Creek, Idaho. For each sample, the numbers of each taxon present and its percentage (in parentheses) of the total sample are given.

Taxon	Hour of sampling (MDT)		
	09:00	15:00	09:00
Ephemerella inermis	191 (70)	32 (59)	166 (50)
Baetis sp.	40 (15)	12 (22)	102 (31)
Other Ephemeroptera	10 (4)	3 (6)	11 (3)
Chironomidae	0	2 (4)	24 (7)
Other Diptera	3 (2)	1 (2)	4 (1)
Other aquatic invertebrates	30 (12)	4 (8)	25 (7)
Terrestrial invertebrates	0	0	0
Total number	274	54	332
N (stomachs)	10	9	10

aggregations in September compared with August, when fish stationed individually. Juvenile anadromous salmonids may also aggregate in groups similar to those in Silver Creek immediately before they leave tributary streams at the onset of winter (Hillman et al. 1987). For resident species, we believe that a brief period of aggregation typically may be the initial signal of the major behavioral and physiological changes that occur at the onset of winter.

Data from Silver Creek study sites are in accord with recent observations suggesting that daytime concealment of juvenile rainbow trout may be initiated at temperatures somewhat warmer than previously believed. Hartman (1965) and Chapman and Bjornn (1969) concluded that juvenile steelhead they observed were concealed at temperatures below 4°C. In small streams in Washington, Campbell and Neuner (1985) found that no age-0 rainbow trout remained in the water column below 6.7°C. In our study, juvenile rainbow trout concealed themselves under the stream bank or in aquatic vegetation during the day at 8.5°C in early October. Later in the day, a few fish were found in the water column when temperatures rose to 13°C. Similar results were reported by Hillman et al. (1992) in small Washington streams, where fewer than 20% of the juvenile salmonids present were visible to snorkelers at temperatures below 9°C. Contor (1989) concluded that a threshold temperature for concealment behavior of juvenile rainbow trout in the Henrys Fork of the Snake River, Idaho, was above 7.5°C.

Daytime and nighttime fish densities declined in both Silver Creek sites during the latter part of September prior to the shift to day concealment behavior. This may have been due to downstream movement or mortality, which we did not monitor, or the initiation of concealment behavior by some individuals before the major shift to day concealment at the end of September. The decline in density of fish counted at night was accelerated at the time of the shift to day concealment, suggesting that some fish might have remained in cover throughout the night as well. Griffith and Smith (1993) found that 61–66% of the day-concealed age-0 brown trout and cutthroat trout (*Oncorhynchus clarki*) were observed at night in enclosed sites in the South Fork of the Snake River, Idaho.

Juvenile rainbow trout in the Silver Creek study area moved into stream margins as light levels dropped after sunset during late summer and, to a greater extent, during winter. This also has been noted in some larger streams, where virtually all age-0 trout observed at night (and electrofished from concealment during the day) were within a few metres of the water's edge (Contor 1989; Schrader and Griswold 1992; Griffith and Smith 1993) as well as in small streams in Washington (Campbell and Neuner 1985). Such behavior appears to be a combined response of selecting positions of low velocity and proximity to cover in lower gradient stream reaches.

In Silver Creek, age-0 trout selected slower and shallower water and moved closer to cover and to the stream bottom at night as temperatures decreased. During winter, they associated closely with vertical and undercut banks and submerged sedges and grasses along the bank. Despite a reduction in its volume during our study period, *Chara* provided concealment cover for trout. However, the decline in fish density by an order of magnitude from August through January suggests to us that winter habitat in our study sites was suboptimal. Furthermore, *Chara* volume in Silver Creek typically continues to decline until March (J.S. Griffith, unpublished data). In other Idaho streams less influenced by groundwater, macrophytes (usually *Myriophyllum* sp.) present in early winter typically slough off during November and December and by midwinter do not hold juvenile rainbow trout (Contor 1989; Schrader and Griswold 1992; Smith 1992). Late winter oxygen depletion in side channel pools has killed trout in British Columbia rivers (Bustard 1986) and might be expected in situations where macrophytes are dense and water movement is less than it was in our study sites.

Invertebrate drift in Silver Creek during fall and early winter was low relative to that in summer, as noted in some Canadian streams by Clifford (1978). However, the quality of that drift was high for juvenile trout. The dominant Silver Creek taxa in October and January were immature mayflies and chironomids, which also formed 80–97% of the winter drift sampled in Convict Creek, California, by Reimers (1957). These groups provided the bulk of the diet of age-0 rainbow trout in summer in the Henrys Fork of the Snake River, Idaho (Angradi and Griffith 1989), as well as in Silver Creek in August.

As expected from previous studies, Silver Creek trout fed during early and midwinter. Fewer than 1% of the stomachs we examined in October and January were empty. Four percent of the brown and rainbow trout stomachs examined in Convict Creek, California, by Maciolek and Needham (1952) were empty. Cunjak and Power (1987) noted that 4.4% of the 6–26 cm fork length brook and brown trout they checked in the Credit River, Ontario, during two winters contained no food. Mean stomach fullness exceeded 50% for the Credit River fish, but that parameter and RWW must be evaluated with caution because of their inflation by a reduced digestive rate in winter.

The period during which age-0 trout shifted to day concealment was transitional in their feeding behavior in Silver Creek. Fish exploited an abundant food source, drifting mayflies and chironomids, during the day and at dusk in August and September, before day concealment began. Similar densities of chironomids in the drift during the daytime in October were not available to fish which were concealed at that time. The nearly significant ($P = 0.06$) negative correlation between weight of food in stomachs and surface drift density in October suggests that feeding (primarily on mayflies at night) occurred when drift was low.

The term anorexia (sensu Mrosovsky and Sherry 1980) has been applied to nonsmolting Atlantic salmon parr during the

winter when they are hidden in the stream bed (Metcalfe and Thorpe 1992). In their laboratory, salmon showed a loss of appetite even in the presence of excess food. If Silver Creek rainbow trout could be identified as anorexic, it would be during the October period when the abundant chironomids during the day could have benefited the Silver Creek juvenile rainbow trout energetically. Their stomach evacuation rate, based on temperatures during our October sample, might have enabled trout to consume two meals per day. Evidently, the perceived risk of predation (or some other factor) overrode the potential benefit of a second (daytime) meal. By January in Silver Creek, trout activity was more synchronized with the availability of those nocturnally drifting mayflies, and RWW and numbers of organisms in trout stomachs increased. Additional sampling during October–January would be required to more thoroughly assess these observations.

Numbers of Silver Creek trout visible in the water column at night in late November peaked at a light level of 0.001 $W \cdot m^{-2}$ at 30–60 min after sunset, a pattern similar to that observed for age-0 rainbow trout during winter by Contor (1989). The numbers of trout observed just after sunset and before sunrise may be indications of most active feeding activity during twilight, but more observations are needed to evaluate this. We occasionally noted trout feeding during our winter night snorkeling, as did Contor (1989). At summer temperatures, rainbow and brown trout fed at night under laboratory conditions at light levels similar to those during our Silver Creek observations (Jenkins 1969; Robinson and Tash 1979). Hatchery-reared age-0 rainbow trout held in Convict Creek, California, by Jenkins et al. (1970) fed on drifting insects, especially *Baetis* sp., during the night in December at 3–6.5°C. Elliott (1973) made similar observations for brown and rainbow trout at 4.7°C.

Benthic rather than drift feeding by Silver Creek trout would have confounded our results. The occurrence of algae, other plant material, or detritus in stomachs often accompanies epibenthic feeding (Tippets and Moyle 1978). We did not find these materials in the stomachs examined. There was some indication of epibenthic feeding at night in October and January, when the amphipod *Gammarus lacustrus* and larvae of the caddisflies *Hydropsyche* and *Oxyethira*, not collected in the drift, were found in a few stomachs. We saw no evidence of benthic feeding during the day in any of the samples. Cunjak and Power (1987) found evidence of the consumption of benthic organisms in early winter by brown trout, but not by brook trout, in the Credit River.

Early winter has been hypothesized as time of metabolic deficit in trout because of the cost of acclimation to decreasing stream temperatures (Cunjak and Power 1987; Cunjak 1988b). Condition factors of brook trout in the Credit River, Ontario, declined markedly from August to the November–December period (Cunjak and Power 1987), and the energy derived from feeding was insufficient to offset the costs of maintenance metabolism. The most substantial loss in energy content of juvenile Atlantic salmon in a Scottish stream occurred during October–December (Gardiner and Geddes 1980).

Water temperature in Silver Creek was moderated by a few degrees, but there was evidence of a metabolic deficit (Riehle 1990) similar to that for trout at Credit River, Ontario, sites where groundwater maintained mean winter temperatures above 3.5°C (Cunjak 1986). Although we cannot rule out the possibility that other factors may be involved, we speculate that the metabolic deficit faced by Silver Creek trout began in September and October. Mean RWW was 1% or less for the trout we sampled in September and October, lower than that in January, and body condition showed the greatest significant decline in September (Riehle 1990). These observations are consistent with those of Smith (1992), who found that 95% of the winter mortality of age-0 rainbow trout in enclosures in the Henrys Fork of the Snake River occurred within the first 7 wk following initiation of daytime concealment. Further studies are needed to fully understand the behavioral and physiological changes that occur during this critical period of transition for stream-dwelling salmonids.

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Use of Oceanographic Forecasts and Echosounders to Guide and Enhance an Inshore Gillnet Fishery for Atlantic Cod (*Gadus morhua*)[1,2]

G.A. Rose[3] and W.C. Leggett

Department of Biology, McGill University, 1205 Ave. Dr. Penfield, Montreal, QB H3A 1B1, Canada

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Vessels fishing with gill nets for Atlantic cod (*Gadus morhua*) in the northern Gulf of St. Lawrence in 1985 and 1986 concentrated their fishing effort in shallow waters (<50 m) where cod densities were highest (to 0.5/m^3). In both years, seasonal trends (June–August) in mean daily deployment depths of gill nets were positively correlated with mean depths at which cod were surveyed (1985, $r = 0.71$; 1986, $r = 0.51$, P's < 0.05). Daily catch rate variability of individual vessels was accounted for by fish "flux" adjacent to nets (44%), vessel operator skill (19%), and "flux"–skill interaction (8%; total $R^2 = 0.71$). A guided vessel directed to fish at sites predicted to have high fish flux (located down-current from high-density cod aggregations identified by echosounding within depth ranges forecast to be favorable to cod by "rule of thumb" wind-based oceanographic models) had higher catch rates (mean 1.3 t/d) than the fleet average and its own average fishing without guidance (means 0.4 t/d, P's < 0.05). Directed searches were of shorter duration (mean 0.5 h) than searches conducted at random (1.5 h). Combined use of echosounders and air–sea-based forecasts of cod distribution could help stabilize catch rates, especially at times of poor fish availability inshore.

Des bateaux qui pêchaient avec des filets maillants la morue franche (*Gadus morhua*) dans le nord du golfe du Saint-Laurent en 1985 et 1986 ont concentré leur effort de pêche dans les eaux peu profondes (<50 m) où la densité des morues était la plus élevée (jusqu'à 0,5/m^3). Les deux années, les tendances saisonnières (juin à août) de la profondeur moyenne de mouillage des filets maillants étaient positivement corrélées à la profondeur moyenne à laquelle la morue avait été repérée (1985, $r = 0{,}71$; 1986, $r = 0{,}51$; $P < 0{,}05$). La variabilité quotidienne dans le taux de capture de chaque bateau s'expliquait par le passage du poisson à côté des filets (44 %), la compétence du patron du bateau (19 %) et l'interaction entre le passage du poisson et la compétence (8 %; total $R^2 = 0{,}71$). Un bateau ayant reçu pour instructions de pêcher sur des sites où on prévoyait que le passage du poisson serait élevé (situés en aval par rapport aux fortes agrégations de morue repérées par échosondage, dans des fourchettes de profondeur jugées favorables à la morue par des modèles océanographiques empiriques fondés sur le vent) a connu un taux de capture plus élevé (moyenne 1,3 t/jour) que la moyenne de la flottille et que sa propre moyenne de pêche sans directives (moyenne 0,4 t/jour; $P < 0{,}05$). Les recherches dirigées duraient moins longtemps (moyenne (0,5 h) que les recherches menées au hasard (1,5 h). L'emploi combiné des échosondeurs et des prévisions fondées sur le vent et la mer de la distribution de la morue pourrait aider à stabiliser les taux de capture, particulièrement dans les périodes où il y a peu de poisson dans les eaux côtières.

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A key problem in the inshore fishery for Atlantic cod (*Gadus morhua*) in Canada is unpredictable variability in the year to year and day to day success rates of its most important gear types, the trap and gill net (Lear et al. 1986; Rose and Leggett 1988a, 1989a). Gaining some control over this variability would be a major advance for these fisheries. Control requires identification and quantification of the key factors that regulate catch rates.

The codtrap is an immobile fishing gear deployed near shore at fixed locations (Whiteley 1977; Rose and Leggett 1988a). In trap fisheries, the potential for control over variability in catch rates is thought to be limited to large spatial scales (>100 km), over periods of several years, through long-term control of stock abundance (Rose 1992a). At smaller spatial and temporal scales (the scale of individual traps to 100's of kilometres; days to a year), control is less likely because variations in trap catches at these scales are largely regulated by variations in the local availability of fish (e.g., Jeffers 1931; KcKenzie 1934; Thompson 1943; Templeman 1966; Lear et al. 1986; Rose and Leggett 1988a, 1989a) and only weakly correlated with variations in overall stock abundance (Lear et al. 1986). The potential for movement of traps to counter variations in fish distribution is very limited. Hence, trap fishermen can do little to improve catches at times of poor fish availability inshore.

The potential to control catch rates in inshore fisheries employing fixed but moveable gear (lines and gill nets) is likely

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[3]Present address: Department of Fisheries and Oceans, Science Branch, P.O. Box 5667, St. John's, NF A1C 5X1, Canada.

to be greater than in trap fisheries. If fish aggregations occur at any location on the fishing ground, mobile gear may be moved there (e.g., Rose and Leggett 1989a). However, simply locating high-density fish aggregations may not assure high catch rates. Environmental factors known to affect fish behavior, and the skill of individual fishermen, can be important contributors to fishing success. For example, gillnet catch rates are strongly correlated not only with local fish aggregation densities, but also with currents in the vicinity of the nets (Rose and Leggett 1989a). This correlation between catch and currents is likely to have occurred because cross-shore movements of cod were positively correlated with the same currents (Rose and Leggett 1988a). It is well known among fishermen that gillnet effectiveness (sensu Dickson 1989) will be low if fish are sedentary (Angelsen and Olsen 1987; G. Labadie and L. Hobbs, commercial fishermen, Brador Bay, Que., personal communications).

We hypothesized that at scales equivalent to gear dimensions, catch rates in a cod gillnet fishery would be positively correlated with the rate of passage, or "flux," of cod adjacent to the nets. We also hypothesized that the skill of individual fishermen would account for a significant portion of the variance in catch rates, independent of fish density or flux.

The control of catch rates in mobile gear fisheries depends on efficient fish location methods. Cod, like most fishes, are most frequently found where environmental conditions (temperatures, depth, salinity, currents, and prey abundance) are within consistent limits (Scott 1982). For example, the average locations (depths) at which cod aggregate daily in the inshore environment of the northern Gulf of St. Lawrence are associated with variations in winds, currents, sea temperatures, and prey abundance (Rose and Leggett 1988a; 1988b, 1989a, 1989b, 1990). These same factors influence cod distributions universally (e.g., Thompson 1943; Hansen 1949; Beverton and Lee 1965; Templeman 1966; Bergstad et al. 1987). Ideally, a search for this species would be confined within boundaries determined by the spatial overlap of the "correct" conditions for all factors. In reality, only one or two factors can practically be considered at a time, and their boundaries can often encompass a large portion of the fishing ground (e.g., Rose and Leggett 1989b, 1990). Within those "acceptable" boundaries, fish aggregations of sufficient density to be of interest to commercial vessels will occur at much smaller scales, either in response to unassessed environmental conditions, or at random (Rose and Leggett 1989b, 1990).

For purposes of fish location and net deployment, environmental conditions are likely to provide only a large-scale and low-resolution map. To pinpoint fish aggregations and guide net deployment, either within zones delimited by environmental conditions or at random, some inshore fishermen use echosounders. However, the majority use traditional placement methods based on historic knowledge of the fishing grounds and conditions combined with trial and error fishing.

In this study, we investigated the fishing strategies and catch rates of inshore gill netters at small temporal (days) and spatial scales (<2 km, equivalent to gear dimensions). We tested the "flux" hypothesis. We then conducted experiments to compare the fishing successes of an acoustic search vessel and "guided" capture with those of other vessels fishing the same grounds. We tested two hypotheses: (1) that acoustic searches for high-density fish aggregations centered at depths predicted by wind-based models (Rose and Leggett 1988a) would be of shorter duration than random searches and (2) that catch rates of gear deployed downstream (relative to predicted currents) from high-density fish aggregations (as determined acoustically) would be greater than catch rates of gear deployed using traditional net placement methods.

Methods

The study site was centered at Brador Bay (51°25′N, 57°15′W) in the northern Gulf of St. Lawrence; its boundaries correspond to the fishing grounds of the gillnet fleet studied. Cod of the northern Gulf of St. Lawrence (NAFO 4RS3Pn) migrate annually to Brador Bay on the Quebec lower north shore in search of their chief prey, capelin (*Mallotus villosus*) (Rose and Leggett 1989b, 1990). Movements of cod to shore are associated with wind-forced upwellings of cool deep saline Gulf waters (Rose and Leggett 1989a). Upwelling conditions dominated coastal hydrographics in 1985, but were weak in 1986, resulting in cod distributions being more shoreward and in shallower waters in 1985 than in 1986 (Rose and Leggett 1988a, 1988b, 1989a, 1989b). Additional details of the site, its physical oceanography, its fisheries, and the factors regulating cod distribution and abundance in the area, are given in Rose and Leggett (1988a, 1988b, 1989a, 1989b, 1990, 1991).

Cod densities were assessed daily from 11 July to 2 August 1985 and 13 June to 4 August 1986 using a 120-kHz Biosonics, Inc. dual-beam (10–25°) echosounder. An average of 50 (range 21–116) randomly selected transects of 1000 m length were surveyed daily during daylight hours. Transects covered all depth ranges of the study site. All density values are referenced to this sampling scale (10 m deep × 1000 m horizontal). Full details are given in Rose and Leggett (1988b, 1988c, 1989a, 1989b). Mean depths where cod were located were weighted by the relative density of cod in each sampling block.

Oceanographic instruments moored at six locations within the study site recorded currents and sea temperatures each 15 min throughout the study. Details of instrument placement and hydrographic dynamics at the study are given in Rose and Leggett (1988b). Currents are referred to by their direction of travel (NE flows NE) and winds by their direction of origin (NE blows from NE).

The gillnet fleet at Brador Bay was monitored daily in both 1985 and 1986. It comprised 30 vessels, each deploying an average of 20 large-mesh (14 cm) monofilament gill nets (90 × 3.6 m) daily. Effort units were gillnet vessel days. The catch rates and net locations (latitude, longitude, and average depth) were documented for an average of 16 vessels selected randomly each day in 1985. In 1986, this information was recorded from 25 vessels preselected on the basis of the consistency (not efficacy) of their fishing effort in 1985.

Totals of 519 and 607 vessel days were monitored in 1985 and 1986, respectively. A subset of 88 fishing locations during 1985 was used to compare catch rates, fish densities, and currents. This subset was determined by the following restrictions: (1) where >1 vessel fished within 1 km^2 (a common occurrence), catch rates were pooled; (2) acoustic fish density estimates were applied only to fishing locations <2 km down-current of the transect; and (3) current strengths and directions were applied only to locations within 4 km of the moored current meter stations (see Rose and Leggett 1988b). For each location, an average index of fishing skill of the vessels involved was assigned. The skill index for each vessel was its daily mean catch over the duration of the study standardized to take a value

TABLE 1. Data from the guided gillnet experiments conducted at Brador Bay in 1986 (winds, currents, search descriptors, and the mean depth at which cod was surveyed). Winds are major directions during the 24-h period prior to net deployment. Currents are nontidal alongshore components predicted by winds (see Methods) and those observed during the period of net deployment. For winds and currents, lowercase indicates weak and uppercase strong relative magnitude. Searches were conducted in shallow (10–30 m) waters following NE currents and deep (30–50 m) waters following SW currents. Gill nets (20) were set down-current from the first located fish aggregation having linear dimensions >100 m and density 0.1/m^3.

	Current			Search			
Day	Wind	Predicted	Observed (m)	Depth (min)	Time (m)	Set (m)	Cod (m)
179	w, se	ne	sw	10–30	10	24	26
180	NE	SW	SW	30–50	45	30	28
181	ne, n	sw	sw	30–50	20	30	30
182	NW	nil	nil	30–50	60	40	52
183	NW	nil	nil	30–50	45	38	36
184	NE	SW	SW	30–50	15	36	34
193	ne, nw	sw	sw	30–50	25	48	53

between 0 and 1 (1 being the highest observed mean catch of a single vessel, 1.2 t).

For each fishing location, fish flux through the area occupied by the nets was calculated. Several assumptions were made: (1) that net deployment time was 24 h, (2) that fish densities were concentrated near the bottom where the nets were anchored, and (3) that fish movement correlated with the alongshore component of the currents (tidal frequencies removed). Assumption 2 was probably valid for most, but not all, of the 24-h period (e.g., Rose and Leggett 1989b; Dickson 1989). Assumption 3 is consistent with the findings of Rose and Leggett (1988a).

We used a path analysis model (Sokal and Rohlf 1981, p. 642) to assess the contributions of colinear variables (densities, flux, and vessel skill) to the explained variations in catch rates. Path analyses assist in separating causal from correlative relationships among variables (e.g., Rose and Leggett 1988a). Standard multiple regression techniques were used to develop a more efficient "predictive" model.

Independent trials of an experiment were conducted on seven days in 1986 (Table 1). In each trial, the alongshore component of the current was forecast. The forecasting "model" was a conditional "rule of thumb" similar to those traditionally used by inshore fishermen: if SW winds occurred for 20 h, then NE-ward currents would result; under NE wind conditions, currents would be SW-ward; if winds were from the NW (off the land) or SE (onshore), then the alongshore component of the current would be nil (Rose and Leggett 1988b). Predicted currents were used to determine the starting depth for the acoustic search for fish (based on the current-depth model given in Rose and Leggett 1988a) and to position nets down-current of fish aggregations.

On the north shore of the Gulf, NE currents are associated with upwelling conditions that bring cod shoreward to shallow waters; SW currents are associated with downwelling of warmed surface waters and movements of cod to deeper waters (Rose and Leggett 1988b). Cod at Brador Bay move to shallower waters following upwelling and to deeper waters following downwelling (Rose and Leggett 1988a). Hence, acoustic searches began in shallow (20 m) waters when NE currents were forecast (only 28 June). On all other days, SW currents were forecast; hence, searches began in deeper (40 m) waters. Searches were terminated when a fish aggregation having linear dimensions >100 m and a density >0.1 m^3 was located. Twenty gill nets were then deployed by a commercial vessel down-current from the fish aggregation. The same search and fishing vessels and crews were used in each trial. On each trial day, the search vessel also ran randomly positioned acoustic transects throughout the study site (for details, see Rose and Leggett 1988a, 1989a).

Results

Cod Distribution and the Fishery

The maximum densities of the cod aggregations surveyed daily increased as cod moved shoreward, at the scales we measured, in both 1985 and 1986 (Fig. 1). This increase was especially noticeable when cod moved very close to shore into waters having depths <30 m. In every case, maximum densities occurred in the sampling block (10 × 1000 m) adjacent to the bottom (all surveys were conducted during daylight hours) (see Rose 1992b for echograms of cod in shallow water). The higher densities nearshore reflected an increase in the area occupied by cod within each transect coupled with an increase in the within-aggregation packing of fish (the mean observed aggregation size in the horizontal plane was less than one sampling unit). To quantify the depth–density relationship, all data were pooled because between-year differences were not significant (Mann–Whitney test, $P = 0.28$). The mean depth at which fish were located accounted for 47% of the variance in the maximum densities surveyed daily in both 1985 and 1986.

Gill nets were deployed over a range of depths between approximately 5 and 90 m in both 1985 and 1986 (Fig. 2). However, within that range, nets were set in deeper waters in 1986 (when upwelling was weak) (mean 31, median 27 m) than in 1985 (mean 18, median 16 m) (χ^2 test, $P < 0.01$).

The mean depth at which gill nets were deployed daily was positively correlated with the mean depth at which cod were surveyed in both 1985 ($r = 0.71$, df = 20, $P < 0.05$) and 1986 ($r = 0.51$, df = 34, $P < 0.05$) (Fig. 3). However, the strength of these correlations was dependent on similar trends (shallower to deeper as the season progressed) in gillnet and cod depths; first-order differences of these variables (Fuller 1976), with net depth lagged 1 d behind fish depth, were less strongly correlated in 1985 ($r = 0.41$, $P = 0.07$) and 1986 ($r = 0.31$, $P = 0.09$). In both years, but especially in 1985, nets were consistently deployed at depths shallower than cod were located.

Low catch rates dominated this fishery in both 1985 and 1986

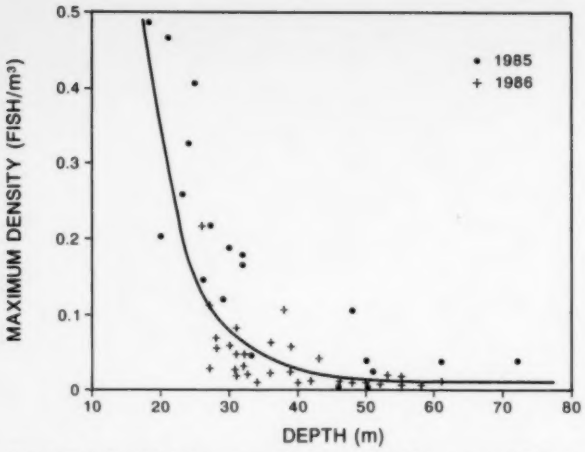

FIG. 1. Relationship between maximum cod densities surveyed daily at Brador Bay (reference volume 10^4 m^3) and average depths occupied by cod in 1985 and 1986. The best fit multiplicative model was $Y = 16.4X^{-2.9}$ ($R^2 = 0.47$).

(Fig. 4). High catch rates (>1 t/d) were rare in both years. The overall ranges of catch rates were 0–4990 kg (mean 503, median 295 kg) in 1985 and 0–3170 kg (mean 474, median 309 kg) in 1986. Catch rate frequency distributions did not differ between years (χ^2 test, $P < 0.01$).

The path analysis model whose structure most closely duplicated the observed correlations among predictor (independent) and criterion (dependent) variables (fish flux, vessel skill, fish density, currents, and catch rates) accounted for 71% of the variance in catch rates at spatial scales equivalent to gear dimensions (<2 km) (Table 2). Of the univariate paths, flux accounted for 44%, and skill 19%, of total variance, independent of other factors (Fig. 5). No other univariate path accounted for more than 1% of the variance. The bivariate path between flux and skill accounted for an additional 8% of the variance. A multiple regression model employing only flux and vessel skill also accounted for 71% of the variance in catch rates (Table 3; Fig. 6). An apparent increase in variance with catch magnitude was noted (we judged the use of transformed data to add little to our interpretations of these data).

Experiments

The directions and relative magnitudes of the alongshore currents predicted by the wind-based "model" were the same as those observed on six of seven days (Table 1). The depths at which the trial nets were set did not differ from the mean depths at which cod aggregated (paired t-test, $P < 0.05$).

The time required to locate a large fish aggregation (linear dimensions >100 m) having high density (>0.1/m^3) was related to the acoustic search method employed. When search patterns were centered around the depths predicted by the wind-based model, mean search times (0.5 h, SE = 0.2) were less than when transects were random with respect to depth (mean 1.5, SE = 0.5) (Mann–Whitney test, $P < 0.05$).

Catch rates of the guided commercial vessel exceeded the mean of the unguided vessels by a factor of 2–3 in each of seven trials (Fig. 7). During the trials, the mean overall catch rate of the guided vessel (1.3 t/d, SE = 0.2, $n = 7$) was significantly greater than that of other vessels (0.4 t/d, SE = 0.1, $n = 105$)

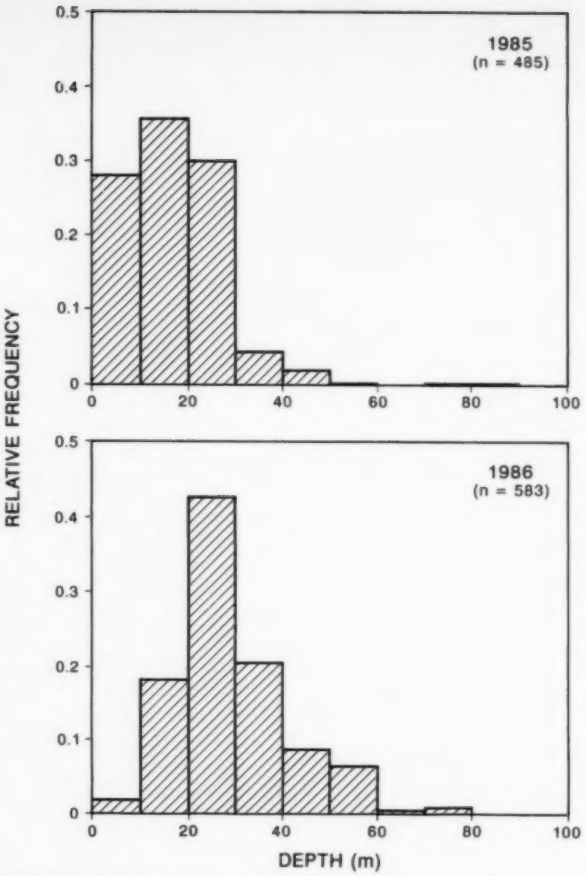

FIG. 2. Relative frequencies of depths of deployment for gill nets set at Brador Bay in 1985 and 1986. Median depths were greater in 1986 (27 m) than in 1985 (16 m) (Mann–Whitney test).

(Mann–Whitney test, $P < 0.05$). On other days, when the crew of the experimental vessel fished without acoustic guidance, their catch rates (mean = 0.4 t/d, SE = 0.1, $n = 19$) were less than on guided days and did not differ from those of the balance of the fleet (mean = 0.5 t/d, SE = 0.1, $n = 591$) (Mann–Whitney tests, P's < 0.05).

Discussion

The Fishery

At Brador Bay, the abundance, densities, and depths at which cod locate themselves during the summer onshore migration vary not only between years, but also daily (Rose and Leggett 1988a, 1989a, 1991). Hence, to conduct a successful gillnet fishery, especially during times of low abundance, fishermen must locate aggregations of these highly mobile fish on a daily basis. Our data indicate that, in general, fishermen are adept at following seasonal trends in the depths of fish distribution, but somewhat less so in tracking higher frequency day to day movements. Thus, despite the considerable skill exhibited by most fishermen, and their intimate knowledge of their fishing grounds, low catch rates dominated the fishery. A great deal of

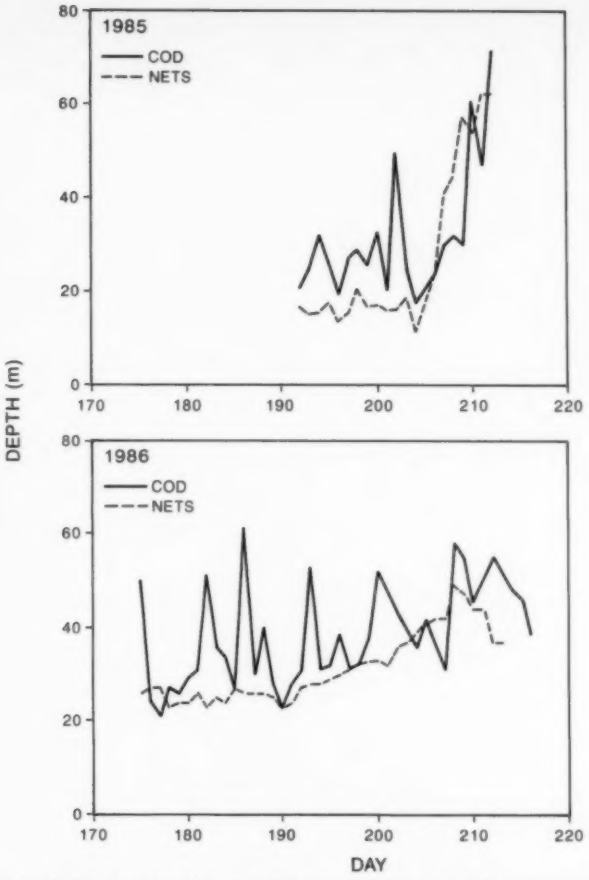

FIG. 3. Relationships between daily mean depths at which cod were located and at which gill nets were deployed at Brador Bay in 1985 and 1986. Trends in fish and net depths were significantly correlated in both 1985 ($r = 0.71$, df = 20) and 1986 ($r = 0.51$, df = 34), but first-order differences (Fuller 1976) were not significantly correlated in either year.

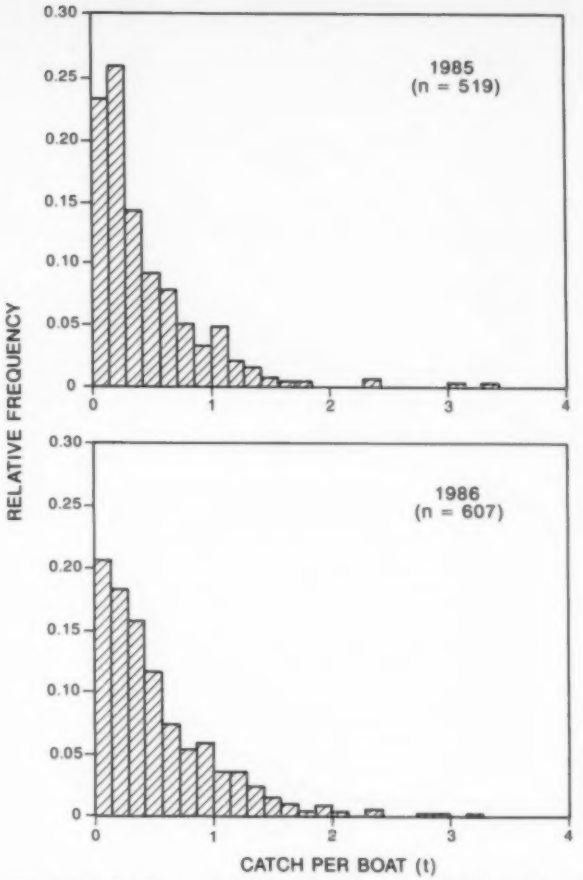

FIG. 4. Relative frequencies of catch rates (per boat) for gillnet vessels at Brador Bay in 1985 and 1986. The single highest catch of 4990 kg in 1985 is not shown.

effort was expended with little success.

Our data indicate that to consistently secure high catches, gillnet fishermen must not only locate fish aggregations on a daily basis, but also predict and then intercept their movements. Fishermen attempt to do this (G. Labadie and L. Hobbs, personal communications). It is instructive to compare the daily depths of net deployments, relative to the depths at which cod were located, between 1985 and 1986. In 1985, fish occurred at much shallower depths during the daytime than in 1986. Moreover, these fish came right to shore during evening feeding forays in 1985, but did not do so in 1986 (further discussions of onshore movement variability are given in Rose and Leggett 1988a, 1989a). Fishermen exploited the shoreward movement in 1985 by deploying nets in very shallow waters where near-bottom fish densities were likely to be highest. In 1986, when shoreward movements were very limited, nets were set in deeper waters adjacent to the daytime locations of the fish. It is evident that this fishing strategy was successful. Catch rates in 1986 were as high as those in 1985 despite the lack of shoreward movement of fish and the much reduced biomass in 1986 (Rose and Leggett 1989a).

Observations at Brador Bay in both 1985 and 1986 indicated that cod concentrate nearshore, possibly in response to the compaction of subthermocline waters having suitable temperatures and salinities (Rose and Leggett 1988a, 1989a) and to the highly aggregated distributions of their capelin prey (Rose and Leggett 1989b, 1990). It is noteworthy that the overall biomass of cod at Brador Bay has been shown to be correlated with the two-dimensional range occupied by the fish (Rose and Leggett 1991). This relationship suggested that cod densities assessed at small scales (1 km) tend to remain relatively stable (within approximately 1 order of magnitude) as compared with variations in the overall biomass of cod migrating into Brador Bay, which range over several orders of magnitude. The present results indicate that much of the variance in density at small spatial scales results from across-shore shifts in cod distribution which in turn are related to upwelling changes in the physical environment (temperatures, salinities, and currents) and to the changing distributions of capelin (Rose and Leggett 1988a, 1989a, b, 1990).

Our data suggest that fishing success rates are highest in shallow waters (<40 m) where cod densities reach their summertime maximum (assuming cod come ashore). Brador Bay fisher-

TABLE 2. Statistics from the path analysis model relating gillnet catch rates (kg/d) at scales equivalent to those of gear dimensions (<2 km), to fish flux (fish·3600 m^{-2}·24 h^{-1}), vessel skill (0–1), fish density (fish/10^4 m^3), and alongshore currents (m/s) near the nets at Brador Bay in 1985.

	Pearson correlation matrix				
	Catch	Flux	Skill	Density	Current
Catch	1.00	0.74	0.70	0.58	0.23
Flux		1.00	0.48	0.86	0.20
Skill			1.00	0.37	0.25
Density				1.00	–0.08

	Standard partial regression coefficients (path coefficients) of catch vs.:
Flux	0.66
Skill	0.44
Density	–0.15
Current	–0.02

	Breakdown of variance of path analysis model (%)
Single variables	
Flux	44
Skill	19
Colinear paths	
Skill–flux	8
Total accounted for	71
Error	29
Total	100

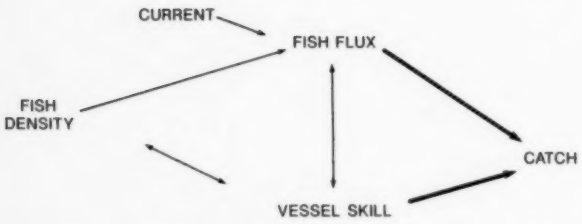

FIG. 5. Schematic diagram representing the path analysis model relating gillnet catch rates (fish/d) at Brador Bay in 1985 to fish flux (fish·3600 m^{-2}·24 h^{-1}), vessel operator skill (0–1), alongshore currents (m/s), and fish densities (fish/10^4 m^3) near the nets. Heavy arrows represent standardized partial regression coefficients which assess effects independent of other factors; light arrows represent simple correlation coefficients. Single arrowheads imply cause and effect; double arrowheads imply simply correlation. All relationships were positive in sign.

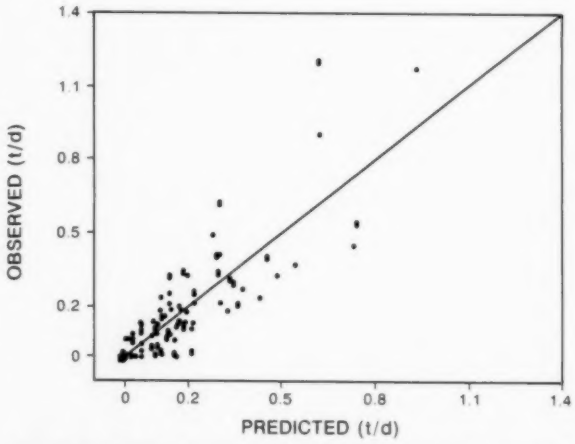

FIG. 6. Relationship between fish catches predicted by the multiple regression model given in Table 2 and those observed (R^2 = 0.71).

men avoid fishing in deeper waters unless there are few fish nearshore; this strategy results both in higher catch rates and in a great reduction in the number of lost nets (G. Labadie and L. Hobbs, personal communications). Norwegian coastal fishermen also avoid deploying gill nets in deeper waters, presumably because cod densities are too low offshore to provide a profitable gillnet fishery (Dickson 1989). An exception to this general practice occurs in the spring fishery for cod off northeastern Newfoundland (Templeman and Fleming 1956). This fishery exploits concentrations of migrating cod inhabiting very deep waters (250–400 m) below the front between cold Arctic waters of the Labrador current and deeper warmer Atlantic waters. This front is believed to constrain cod migration (Lear et al. 1986) because its temperatures (less than –1°C) are below the range (–0.5 to 8.5°C) within which cod are typically found (Rose and Leggett 1989b). More recent studies conducted seaward from the front have shown that cod migrating shoreward tend to hold constant temperatures and avoid thermal gradients (Rose 1993). Hence, cod may become temporarily concentrated at the front (as they do when approaching the coast). Resultant increases in local densities and fish fluxes may underpin the success of this gillnet fishery, as they do in shallower waters.

The path analysis model indicated that gillnet catch rates are determined to a large extent by the flux of fish across the nets.

TABLE 3. Statistics of a multiple regression model of gillnet catch rates (kg/d) at the scales of gear dimensions (<2 km) and as a function of fish flux (fish·3600 m^{-2}·24 h^{-1}) and vessel skill (0–1).

Dependent variable: catch rate, $R^2 = 0.71$ (adjusted for df)			
Predictor	Coefficient	t	P
Flux	0.5	7.88	<0.001
Skill	470.3	6.63	<0.001
Constant	–33.0	–1.34	0.18

ANOVA for full regression					
Source	Sum of squares	df	Mean square	F	P
Model	3066806	2	1533404	101	<0.001
Error	1284901	85	15116		

This result confirms that catch rates may be low even when fish densities adjacent to the nets are relatively high if currents are weak or, for other reasons, fish are not moving. We conclude that to maximize catch rates, gill nets should be placed across the flow (as much as possible) and down-current from fish aggregations, at least during the spring–summer period when cod are moving with currents. We note that this strategy may at times be impractical at sites where cod aggregate but currents are strong (e.g., coastal tidal stream zones) (G. Labadie, personal communication). In these cases, it may be feasible to deploy nets either at some small angle off the axis of the dominant flow or at adjacent sites where flows are less strong.

The path model also supports earlier conclusions that gillnet catch rates are unlikely to relate to larger scale average fish densities (Crecco and Savoy 1985; Angelsen and Olsen 1987; Dickson 1989; Rose and Leggett 1989a). This conclusion is further supported by the observations of fishermen, who hold that mobile gear catch rates are determined for the most part by the extent of fish aggregation on the fishing grounds (not average densities) and the abilities of fishermen to locate these aggregations (L. Hobbs and G. Labadie, personal communications).

The path analysis verified the hypothesis that skill levels of the operators of vessels using mobile gear contribute to catch rate variance independent of fish density or flux. The correlation between skill, density, and flux is thought to reflect the abilities of fishermen to locate high-density fish aggregations and to intercept their movements (e.g., Rose and Leggett 1989a). However, fishing skill does not solely reflect abilities to locate fish. The multiple regression model suggests that, on average, less skilled operators will catch <50% of fish located and available to their nets. Under the same conditions, more skilled operators may be expected to have much higher catch rates (>90%). Moreover, low-skilled operators only rarely secured high catches. Even the most skilled operators at times had low catch rates.

Experiments

Our results indicate that search times may be decreased, and catch rates controlled, by the use of oceanographic forecasts and echosounders. It is noteworthy that although the mean catch rate of the experimental gillnet vessel was several times higher when guided than when not guided, the guided catch rate (1.3 t/d) was no better than that obtained by the operator of the most skilled local vessel (1.2 t/d).

The accuracy with which our wind-based "model" forecast

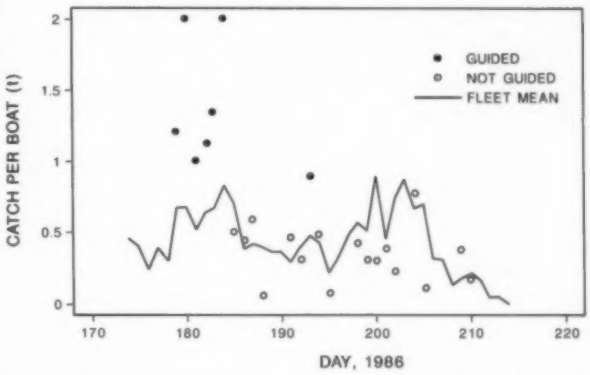

FIG. 7. Mean catch rates (per boat) of the gillnet fishery conducted at Brador Bay in 1986 and those of the commercial vessel guided by oceanographic forecasts and echosounder searches for high-density fish aggregations.

currents within the study area confirms the close association between alongshore winds and currents on the north shore of the Gulf (Rose and Leggett 1988b). These results indicate that useful forecasting of fish distributions may be based on very simple "rule of thumb" models.

The use of empirically derived air–sea "rules" to predict general fishing conditions is traditional among inshore fishermen. We believe this tradition underpins the fishing strategies of the most successful fishermen. For example, on the lower north shore of the Gulf of St. Lawrence, fishermen use winds to forecast general fish distribution. The "broom" (English) or "brume" (French) refers to a brisk wind from the NE (alongshore) which "sweeps" fish from the coast — good fishing will not be found nearshore until SW winds prevail (I. Roberts, commercial fisherman, St. Paul's River, Que., personal communication). In previous studies, we have shown that NE winds are followed by the onshore transport of heated surface waters from the northeastern Gulf or cooler lower salinity waters from the Strait of Belle Isle (Rose and Leggett 1988b). The intrusion of these waters is followed by offshore displacement of cod (Rose and Leggett 1988a, 1989a, 1989b). This conclusion is supported by tagging studies reported by Thompson (1943), who wrote that "the Gulf type of cod ... appear to be very much restricted to their own zone of peculiar hydrographic conditions. They do not extend

their range much beyond the Strait of Belle Isle, appearing to avoid direct contact with Arctic water not only in this region but in the direction of Fortune and Placentia Bay on the south coast of Newfoundland."

Our results indicate that echosounders can greatly assist inshore cod fishermen in locating fish aggregations, especially if used in conjunction with air–sea-based forecasts of probable fish location. This is hardly surprising. Indeed, in the offshore fisheries of the northwest Atlantic (or other oceans), fishing would be uneconomical without the use of echosounders (Laevastu and Hayes 1981). Similarly, in the inshore fisheries for pelagic species such as Atlantic herring (*Clupea harengus*) and capelin, purse-seiners are dependent on echosounders and side-scanning sonar to locate fish. Relatively inexpensive and portable echosounders are now available for the smallest fishing vessel and could be used either by single vessels or cooperatively. The chief constraint on inshore catches is the availability of fish (Rose 1992a). However, echosounders and simple forecasts could greatly assist in stabilizing inshore catches, especially when the availability of fish is poor.

Acknowledgments

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A Reappraisal of Phenotypic Variation in *Daphnia galeata mendotae*: The Role of Interspecific Hybridization

Derek J. Taylor and Paul D.N. Hebert

Department of Zoology, University of Guelph, Guelph, ON N1G 2W1, Canada

Taylor, D.J., and P.D.N. Hebert. 1993. A reappraisal of phenotypic variation in *Daphnia galeata mendotae*: the role of interspecific hybridization. Can. J. Fish. Aquat. Sci. 50: 2137–2146.

Seasonal, within-lake, and among-lake variation in helmet size and shape has been reported for *Daphnia galeata mendotae*. The genetic component of this variation was assessed by an allozyme and morphological reanalysis of nine previously studied sites. Genetic associations with morphological variation were found, but all were attributed to *D. galeata mendotae* × *Daphnia rosea* hybridization or to taxonomic confusion with *D. rosea*. The existence of hybrids accounted for spatial polymorphism both within and among lakes. In addition, hybrids contributed to seasonal variation as they increased in abundance from summer to winter. Nevertheless, cyclomorphosis in *D. galeata mendotae* s.s was real and due largely to phenotypic plasticity. Local hybridization between *D. galeata mendotae* and *D. rosea* was widespread from the midwestern states to Vermont. The distributions and habitat associations of *D. galeata mendotae* and *D. rosea* indicated that ecological segregation at the lake level acts as an important reproductive isolating mechanism. Reinterpretation of previous studies, however, suggests that disturbance followed by one or a few hybridization events often leads to hybrid domination of a lake.

On a signalé une variation saisonnière, au sein d'un lac et d'un lac à l'autre, dans la taille et la forme de la capsule céphalique chez *Daphnia galeata mendotae*. Nous avons évalué la composante génétique de cette variation en étudiant un allozyme et en effectuant une deuxième analyse morphologique de neuf sites étudiées antérieurement. Nous avons découvert des associations entre l'aspect génétique et la variation morphologique, mais elles ont toutes été attribuées à une hybridation de *D. galeata mendotae* × *Daphnia rosea* ou à une confusion taxinomique avec *D. rosea*. L'existence d'hybrides expliquait le polymorphisme spatial tant dans un lac que d'un lac à l'autre. De plus, les hybrides contribuaient à la variation saisonnière du fait que leur abondance augmentait de l'été à l'hiver. Cependant, le cyclomorphisme chez *D. galeata mendotae* s.s. était réel, et dû principalement à la plasticité du phénotype. L'hybridation à caractère local entre *D. galeata mendotae* et *D. rosea* était répandue des États du Midwest au Vermont. Les répartitions et les associations en matière d'habitat de *D. galeata mendotae* et *D. rosea* indiquaient que la ségrégation écologique au niveau du lac constitue en mécanisme important d'isolement pour la reproduction. La réinterprétation des études précédentes semble toutefois indiquer qu'une perturbation suivie par un ou quelques événements d'hybridation conduit souvent à une domination d'un lac par les hybrides.

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Striking morphological variation is common in aquatic invertebrate species. Often, the variation involves defensive structures (e.g., helmets and spines) whose presence is strongly correlated with predation risk (Havel 1987). In cladocerans, the most prevalent pattern of variation is cyclomorphosis — a temporal succession of morphs. Other polymorphic systems lack temporal succession but the morphs are spatially segregated either within water bodies (Green 1967; Zaret 1972) or among water bodies (Riessen and O'Brien 1980).

The freshwater cladoceran *Daphnia galeata mendotae* Birge, 1918 is exceptional in that it exhibits all three of the major polymorphism patterns. The two common morphs are characterized by their differing head size and shape: tall and pointed, and low and rounded. Usually, temporal shifts in the frequencies of these morphs lead to cyclomorphosis, as individuals with tall, pointed helmets are prevalent in the summer and individuals with low, rounded helmets are dominant in the winter (Brooks 1957). Yet, it has been known since Herrick (1884) and Birge (1892) that among-lake spatial polymorphism also exists in *D. galeata mendotae*, as some lakes contain only rounded-head forms during the warm months. In addition, some lakes contain both the rounded- and pointed-head morphs during the summer (Brooks 1964; Malone and McQueen 1983). In these lakes, Brooks (1964) described spatial segregation with the pointed-head morphs dominating the epilimnion and the rounded-head morphs dominating the deeper strata.

Such polymorphism warrants genetic analysis. The initial investigators of allozyme and morphological variation in *D. galeata mendotae* failed to provide evidence of a strong genetic component to these polymorphisms; instead, phenotypic plasticity was thought to account for most of the observed morphological variation (Jacobs 1961; Brooks 1964; Mort 1986; Stirling and McQueen 1987; Mort 1989). Recently, however, Taylor and Hebert (1992) have shown that interspecific hybridization contributes to all three patterns of polymorphism in *D. galeata mendotae*. Their allozyme analyses showed that interspecific hybrids between *D. galeata mendotae* and *Daphnia rosea* are common in the lakes of Indiana. These hybrids, which always possessed a rounded-head phenotype, often cooccurred and underwent seasonal succession with one parent taxon while

TABLE 1. Summary of morph and allozyme phenotypes from prior studies of *D. galeata mendotae* populations. The "intermediate" and "pointed" helmet morphs of Stirling and McQueen (1987) have been pooled in this table under the angulated/pointed category. Parentheses indicate genotype designations of Stirling and McQueen (1987). Enzyme abbreviations are those recommended by IUBNC (1984).

Lake	Prior studies	Midsummer helmet phenotype (%)			Multilocus genotype frequencies					Our taxonomic reevaluation
		High, angulated pointed	High, rounded	Low, rounded	*AO*	*sAAT*	*GPI*	*PGM-2*		
Bantam Lake, Conn.	Brooks 1947; Jacobs 1961	100	0	0	—	—	—	—		*D. g. m.*
East Twin Lake, Conn.	Brooks 1964; Zaret 1972	95	—	—	—	—	—	—		*D. g. m.*
		—	—	5	—	—	—	—		*D. g. m.* × *D. r.*
Mt. Tom Pond, Conn.	Brooks 1964	2	—	—	—	—	—	—		*D. g. m.*
		—	—	98	—	—	—	—		*D. g. m.* × *D. r.*
Queechy Lake, N.Y.	Brooks 1964	0	0	100	—	—	—	—		*D. g. m.* × *D. r.*
Lake Mitchell, Vt.	Mort and Jacobs 1981; Mort 1986	0	0	100	—	—	*ss*	—	0.024	*D. catawba*?
		0	0	100	—	—	*fs*	—	0.803	*D. g. m.* × *D. r.*
		0	0	100	—	—	*ff*	—	0.173	*D. r.*
Lake Morey, Vt.	Mort and Wolf 1985; Mort 1986, 1989	100	0	0	—	*ss*	—	—	0.763	*D. g. m.*
		100	0	0	—	*sf*	—	—	0.237	*D. g. m.*
Haynes Lake, Ont.	Stirling and McQueen 1987	0	100	0	*fs*	*mm*	*mm*	*sf*	1.000	*D. g. m.* × *D. r.*
Lake St. George, Ont.	Malone and McQueen 1983; Stirling and McQueen 1987	88	0	12	(1) *ff*	*mm*	*mm*	*ss*	0.340	*D. g .m.*
		86	0	14	(2) *ff*	*mm*	*mm*	*sf*	0.100	*D. g. m.*
		0	0	100	(3) *fs*	*mm*	*mm*	*sf*	0.100	*D. g. m.*× *D .r.*
		71	0	28	(9) *ff*	*mm*	*sm*	*ss*	0.140	*D. g. m.*
		98	0	2	(15) *ff*	*mm*	*mf*	*ss*	0.100	*D. g .m.*
Three-Lakes-Three, Mich.	Tessier et al. 1992	0	100?	?	—	—	—	—		*D. r.*

the other parent taxon was absent or rare. These abundance patterns generated seasonal changes in morphology that mimicked cyclomorphosis. Among-lake spatial polymorphisms occurred during the summer because some lakes contained only hybrids (with rounded helmets) whereas other lakes contained only *D. galeata mendotae* (with pointed helmets). When both hybrids and *D. galeata mendotae* coexisted within a lake, a dimorphic pattern resulted.

Hybrids between *D. galeata mendotae* and *D. rosea* in Indiana were detected in 17 of the 22 Indiana lakes studied. Given that these species are neighbouringly sympatric (sensu Grant 1981) throughout much of North America, it is unlikely that hybridization is restricted to Indiana. Allozyme diagnosis of hybrids is straightforward because both fixed allelic differences and marked gene frequency divergences exist between their parent species. The present study reevaluates previous reports of phenotypic polymorphisms in *D. galeata mendotae* from lakes in eastern North America and assesses the role of interspecific hybridization as a source of this variation. To our knowledge, we have included lakes from all prior studies that examined the extent of morphological and genetical variation in "*D. galeata mendotae*" at the population level. We provide allozyme and morphological evidence that both *D. galeata mendotae* × *D. rosea* hybridization and misidentification of *D. rosea* are important in accounting for spatial (both within and among lakes) and seasonal patterns of phenotypic diversity in these populations.

Materials and Methods

Populations of *D. galeata mendotae* and *D. rosea* from Indiana (Taylor and Hebert 1992) were used as genetic reference material for the present study. Additional samples of typical *D. rosea* were collected from both Ontario (Guelph) and Vermont (Lake Mitchell) for evaluation of regional variation in allozyme markers. Prior work on morphological and genetic diversity in populations of *D. galeata mendotae* has been limited to nine sites in eastern North America (Table 1). *Daphnia* were collected from these lakes between June 14 and July 9 in either 1990 or 1992. Two of these lakes were sampled again on April 17, 1991 (Bantam Lake and Mt. Tom Pond), for genetic assessment of temporal polymorphisms. Duplicate vertical hauls were conducted with a 30-cm-diameter plankton net (200-μm mesh) and the resulting *Daphnia* samples were frozen in liquid nitrogen. *Daphnia* species were identified according to Brooks (1957).

Electrophoretic Analysis

Individuals of the *D. galeata mendotae* complex were characterized under a dissecting microscope according to helmet shape (round, angulated, pointed) and size (low, high). Cellulose acetate electrophoresis was then conducted using standard methods (Hebert and Beaton 1989). Ten variable loci were used including the following nine loci that Taylor and Hebert (1992) found useful in separating *D. galeata mendotae* from *D. rosea*; aldehyde oxidase (*AO*, 1.2.3.1) aspartate aminotransferase

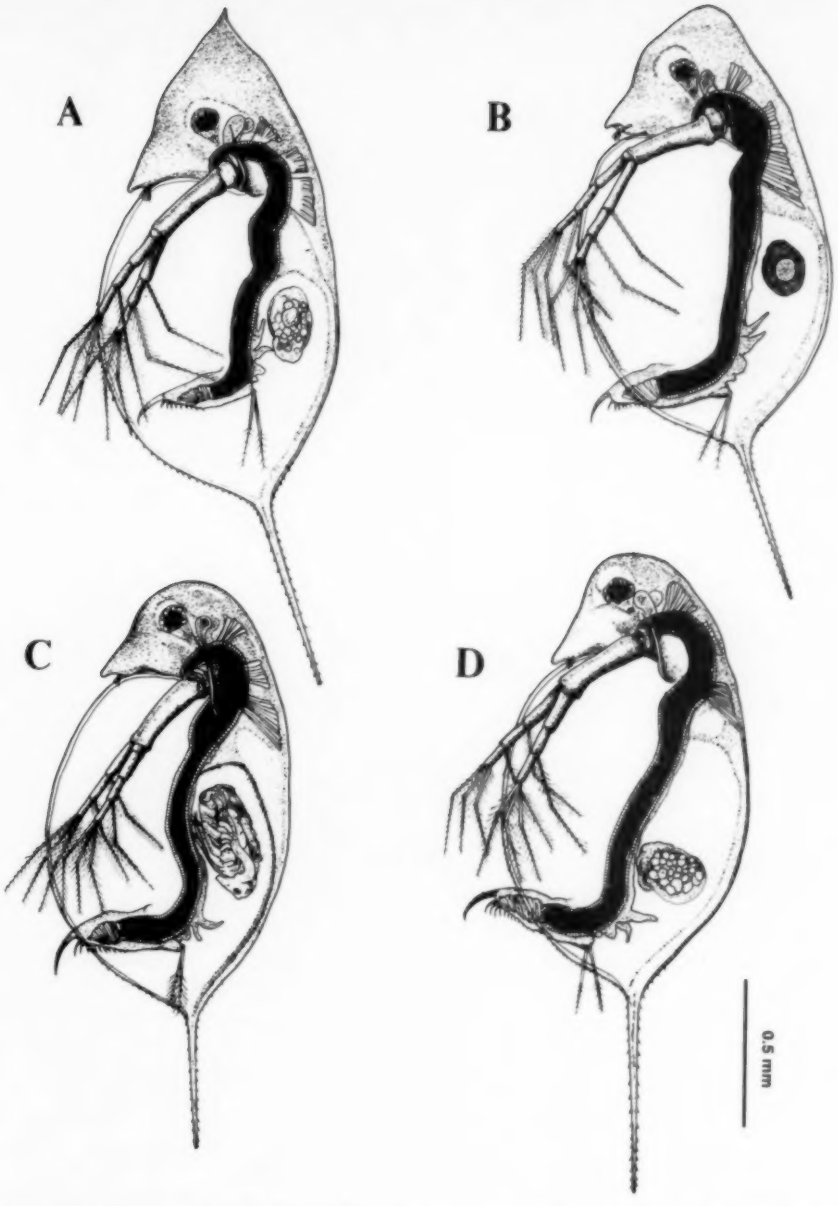

FIG. 1. Midsummer morphs of *D. galeata mendotae*. (A) tall, pointed morph from Lake St. George, Ont., June 19, 1990; (B) tall, angulated morph from same sample; (C) short, rounded morph from same sample; (D) short, rounded morph from Lake Mitchell, Vt., July 9, 1990. Illustrations represent adult females in lateral view.

(*sAAT, mAAT,* 2.6.1.1), dipeptidase (*PEPA*, 3.4.13.11), fumarate hydratase (*FUM*, 4.2.1.2), lactate dehydrogenase (*LDH*, 1.1.1.27), phosphoglucomutase (*PGM-2*, 5.4.2.2), phosphoglucoisomerase (*GPI*, 5.3.1.9), and proline dipeptidase (*PEPD*, 3.4.13.9). Reinterpretation of phenotypic diversity at an additional locus (*PGM-1*) provided another diagnostic difference between *D. galeata mendotae* and *D. rosea* and this locus was also scored in this study. Substrates for *PEPA* and *PEPD* were L-leucylglycine and L-phenylalanyl proline, respectively. Alleles were assigned ascending letter designations with the slowest mobility (relative to the anode) assigned to "a". Individuals from clonal laboratory cultures of typical *D. galeata mendotae* and *D. rosea* from Indiana were used as electrophoretic standards. Nonmetric multidimensional scaling (MDS) of a Cavalli-Sforza and Edwards (1967) chord distance matrix was used to examine the genetic intermediacy of putative hybrid populations.

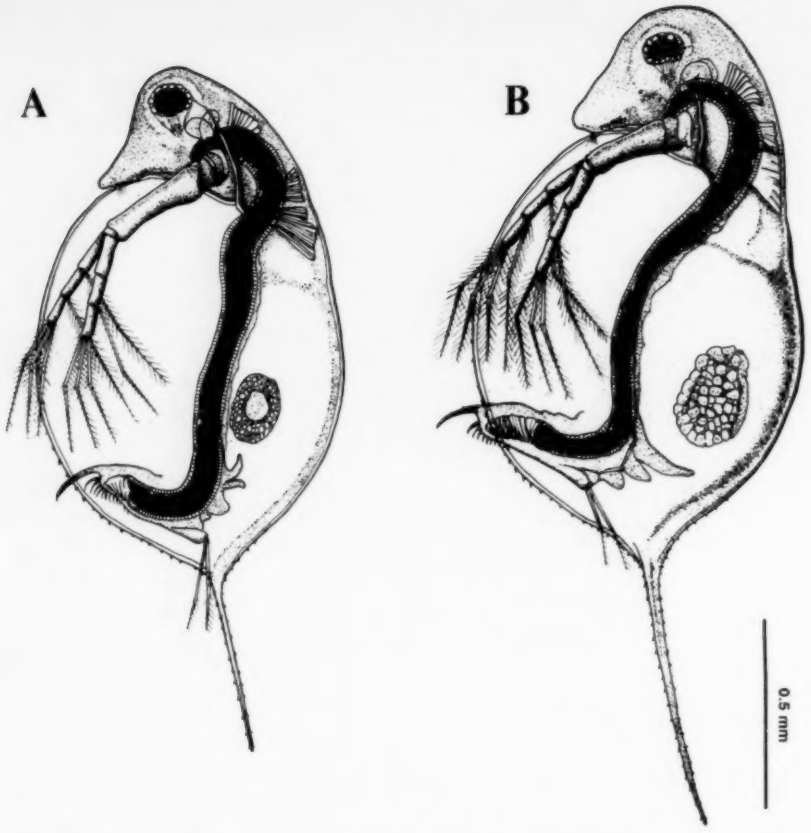

FIG. 2. (A) Mature female of *D. rosea* from Lake Mitchell, Vt., July 9, 1990; (B) mature female of short, rounded morph of *D. galeata mendotae* from Mt. Tom Pond, Conn., April 17, 1991.

Results

Morphological Analysis

The *D. galeata mendotae* s.l. in our samples were assigned to three morphs on basis of helmet size and shape. Individuals possessed either tall, pointed helmets (Fig. 1A), tall, angulated helmets (Fig. 1B), or short, smoothly rounded helmets (Fig. 1C and 1D). *Daphnia galeata mendotae* were absent in our samples from Queechy Lake was collected from the other eight lakes that have been the subject of prior studies. Haynes Lake and Three-Lakes-Three samples contained specimens with low, rounded heads. Our samples from Lake Mitchell also contained a low-, rounded-helmet morph of *D. galeata mendotae* s.l. (Fig. 1D) in addition to *D. rosea* (Fig. 2A). Lake St. George contained all three morphs, while the remaining midsummer samples (Bantam, East Twin, Morey, and Mt. Tom) consisted entirely of specimens with tall, pointed helmets. In contrast, April samples of Bantam Lake and Mt. Tom Pond consisted entirely of *Daphnia* with short, smoothly rounded helmets (Fig. 2B).

Allozyme Analysis

The supplemental reference populations of eastern *D. rosea* (Lake Mitchell and Guelph) possessed similar allelic arrays to those of conspecific Indiana populations (Table 2). They differed from the Indiana populations of *D. rosea* only by the absence of LDH^a and the presence of $PGM\text{-}2^c$ and $PGM\text{-}2^f$. Likewise, many individuals from Haynes Lake and Three-Lakes-Three exhibited allozyme profiles that were typical of *D. rosea* (Table 3). Nevertheless, 61% of Haynes Lake and 15% of Three-Lakes-Three individuals were either backcrosses or advanced generation hybrids, as they possessed either the AO^b or $PEPA^c$ alleles that are diagnostic of *D. galeata mendotae*. Morphs with high, pointed, or angulated helmets (Bantam, East Twin, St. George, Morey, and Mt. Tom) possessed allelic arrays typical of *D. galeata mendotae* (Table 2). Only the *sAAT* locus showed marked gene frequency differences between Indiana and these test populations of *D. galeata mendotae*. Some alleles not detected in *D. galeata mendotae* from Indiana were, however, present in the test populations (GPI^a, $PGM\text{-}2^a$, LDH^b, LDH^d, and $PEPA^b$).

Low-, rounded-helmet *D. galeata mendotae* s.l. from Lake St. George and Lake Mitchell possessed allozyme patterns that are consistent with *D. galeata mendotae* × *D. rosea* hybridization (Tables 2 and 3). Specifically, fixed heterozygosity patterns were observed at *AO*, *mAAT*, and *PGM-1* for alleles that are unique markers of the parental genomes. In addition, high heterozygosity was present at other loci (i.e., *PEPD*, *PGM-2*, and *PEPA* in Lake St. George and *PEPD*, *PGM-2*, and *GPI* in Lake Mitchell). Such heterozygotes consisted of alleles that differ

TABLE 2. Allelic frequency comparison at 10 polymorphic loci among Indiana *D. galeata mendotae* (mean of eight populations), Indiana *D. rosea* (nine populations), eastern *D. rosea* (two populations), and *D. galeata mendotae* reexamined in this study. Numbers in parentheses are sample sizes. ND, no data available.

Locus	Alleles	*D. galeata* (Indiana)	*D. rosea* (Indiana)	*D. rosea* (eastern)	Bantam	East Twin	Morey	Mt. Tom	St. George
mAAT	*a*	— (269)	1.00 (291)	1.00 (178)	— (37)	— (26)	— (67)	— (99)	— (117)
	b	1.00	—	—	1.00	1.00	1.00	1.00	1.00
sAAT	*a*	0.20 (321)	1.00 (669)	1.00 (154)	0.9 (154)	0.90 (195)	0.92 (174)	0.96 (119)	1.00 (138)
	b	0.80	—	—	0.03	0.10	0.08	0.04	—
AO	*a*	— (418)	1.00 (900)	1.00 (184)	— (154)	— (175)	— (132)	— (110)	— (145)
	b	1.00	—	—	1.0	1.00	1.00	1.00	1.00
FUM	*a*	0.30 (336)	— (484)	— (110)	— (176)	— (175)	— (132)	— (110)	— (66)
	b	0.70	1.00	1.00	1.00	1.00	1.00	1.00	1.00
GPI	*a*	— (249)	— (775)	— (243)	— (154)	0.03 (176)	0.24 (196)	— (110)	0.03 (180)
	b	0.96	1.00	1.00	1.00	0.97	0.76	1.00	0.90
	c	0.04	—	—	—	—	—	—	0.07
LDH	*a*	— (430)	0.11 (981)	— (200)	— (65)	— (172)	— (44)	— (110)	— (125)
	b	—	—	—	0.03	0.15	—	—	—
	c	1.00	0.89	1.00	0.97	0.79	1.00	1.00	1.00
	d	—	—	—	—	0.06	—	—	—
PEPA	*a*	0.07 (291)	1.00 (291)	1.00 (182)	— (175)	— (114)	0.03 (141)	0.16 (93)	— (179)
	b	—	—	—	—	0.35	0.02	0.03	0.08
	c	0.93	—	—	1.00	0.65	0.95	0.81	0.92
PEPD	*a*	0.81 (289)	— (301)	— (207)	0.55 (122)	0.98 (142)	0.75 (186)	1.00 (98)	0.97 (188)
	b	0.19	1.00	1.00	0.45	0.02	0.25	—	0.03
PGM1	*a*	— (87)	1.00 (209)	1.00 (140)	ND	ND	— (32)	ND	— (31)
	b	1.00	—	—			1.00		1.00
PGM2	*a*	— (296)	— (876)	— (106)	0.07 (152)	0.02 (192)	0.05 (131)	0.46 (109)	— (191)
	b	0.60	—	—	0.57	0.72	0.90	0.41	0.80
	c	—	—	0.05	—	—	—	—	—
	d	0.40	0.90	0.33	0.36	0.26	0.05	0.13	0.20
	e	—	0.10	0.41	—	—	—	—	—
	f	—	—	0.21	—	—	—	—	—

greatly in frequency between *D. rosea* and *D. galeata mendotae*. Genotypic diversity of the hybrids was low, as only one multilocus genotype was found in Lake Mitchell whereas one hybrid genotype constituted 96% of the Lake St. George sample. No backcrosses or introgressants were detected in either lake.

Both of these hybrid populations possessed the allelic arrays that are expected of hybridization involving local populations of parent species. For example, the Lake Mitchell hybrids had the Pgm^c allele which was detected only in Lake Mitchell *D. rosea*. In addition, alleles that were found in eastern hybrids of this study (GPI^a and GPI^c) were detected in nearby parent populations but not in Indiana *D. galeata mendotae* or *D. rosea*. Similarly, the FUM^a and LDH^a alleles were found in Indiana parent species and hybrids (Taylor and Hebert 1992) but were absent in both hybrid and parent populations of Ontario and New England. Finally, the possession of the $sAAT^{aa}$ genotype by all hybrids in this study also supports the notion of local hybridization, as nearby *D. galeata mendotae* were nearly fixed or fixed for $sAAT^a$ whereas *D. galeata mendotae* in Indiana were nearly fixed for $sAAT^b$.

A spatial summary of these genetic relationships was provided by MDS (Fig. 3). Populations of F_1 hybrids from Indiana were intermediate to their parental populations (Indiana *D. rosea* and *D. galeata mendotae*) in dimension 1. We therefore interpreted this dimension as a hybrid index containing most of the among-species genetic variation. We interpreted dimension 2, on the other hand, as representing genetic variation within taxa because the Indiana populations were separated from their eastern populations. In dimension 1, Lake Mitchell and Guelph *D. rosea* were grouped with Indiana *D. rosea*, while the *D. galeata mendotae* with high, pointed helmets (Bantam, East Twin, St. George, Morey, and Mt. Tom) grouped with the Indiana *D. galeata mendotae*. Dimension 1 showed the genetic intermediacy of the Lake Mitchell and Lake St. George hybrid populations between *D. rosea* and *D. galeata mendotae*. Similarly, Haynes Lake, which contained several introgressants, was intermediate between *D. rosea* and hybrid populations. In dimension 2, the Lake Mitchell and Lake St. George hybrid populations grouped with the eastern populations of parent species whereas 13 of 14 Indiana hybrid populations grouped with Indiana populations of parent species.

Allozyme–Morph Covariation within *D. galeata mendotae* s.s.

The two high-helmeted morphs (angulated and pointed) in Lake St. George, which possessed typical *D. galeata mendotae* allelic arrays, were genetically different. A total of 17 multilocus genotypes (hereafter clones) were apparent from the analysis of four variable loci. Two clones, which differed only at *PGM-2*,

TABLE 3. Genotype frequencies at 10 polymorphic loci in low-, rounded helmet morphs. Bantam and Mt. Tom samples are from April whereas all other lake samples are from midsummer. n = sample size.

Locus	Observed genotypes	Lake St. George	n	Lake Mitchell	n	Mt. Tom	n	Bantam	n	Three-Lakes-Three	n	Haynes Lake	n
mAAT	*aa*	—	55	—	65	—	66	—	49	1.000	33	1.000	42
	ab	1.000		1.000		—		—		—		—	
	bb	—		—		1.000		1.000		—		—	
sAAT	*aa*	1.000	60	1.000	35	0.984	63	1.000	55	1.000	33	1.000	44
	ab	—		—		0.016		—		—		—	
AO	*aa*	—	57	—	107	—	62	—	54	0.850	33	0.425	40
	ab	1.000		1.000		—		—		0.150		0.500	
	bb	—		—		1.000		1.000		—		0.075	
FUM	*bb*	1.000	34	1.000	9	1.000	22	1.000	22	1.000	33	1.000	44
GPI	*ab*	—	66	1.000	117	—	62	—	33	—	33	—	44
	bb	0.955		—		1.000		1.000		1.000		1.000	
	bc	0.045		—		—		—		—		—	
LDH	*cc*	1.000	52	1.000	95	1.000	62	1.000	54	1.000	33	1.000	44
PEPA	*aa*	—	62	1.000	78	—	51	—	32	1.000	33	0.386	44
	ac	1.000		—		—		—		—		0.477	
	bc	—		—		0.098		—		—		—	
	cc	—		—		0.902		1.000		—		0.137	
PEPD	*aa*	—	66	—	105	0.850	60	—	20	—	33	—	
	ab	1.000		1.000		0.134		0.900		—		—	
	bb	—		—		0.016		0.100		1.000		1.000	
PGM-1	*aa*	—	13	—	40	—	22	—	11	1.000	33	1.000	44
	ab	1.000		1.000		—		—		—		—	
	bb	—		—		1.000		1.000		—		—	
PGM-2	*ab*	—	66	—	110	0.742	62	0.039	52	—	33	—	44
	ad	—		—		0.016		0.019		—		—	
	bb	—		—		0.242		0.231		—		—	
	bc	—		1.000		—		—		—		—	
	bd	0.970		—		—		0.346		—		—	
	dd	0.030		—		—		0.365		1.000		1.000	

constituted 68% of the sample. The two alleles we detected at this locus were probably the same as the "*s*" and "*f*" alleles of Stirling and McQueen (1987), as these were the only common alleles found in *D. galeata mendotae* s.s. (Table 2). Helmet morphology differed between these two clones (G-test of independence = 15.27, df = 1, $P < 0.005$), as one (*PGM-2*^{bb}) was associated with a pointed helmet (46 pointed versus 21 angulated) and the other (*PGM-2*^{bd}) was associated with an angulated helmet (18 pointed versus 36 angulated). The same relationship was apparent when the 15 less common clones were pooled into *PGM-2* groups (*bb* or *bd*). *PGM-2*^{bb} clones (28 pointed versus 14 angulated) were more likely to possess pointed helmets than *PGM-2*^{bd} clones (3 pointed versus 13 angulated), ($G = 11.21$, df = 1, $P < 0.005$). Overall, 68% of the *PGM-2*^{bb} individuals had pointed helmets. No significant relationships existed between the other loci and helmet morphology for the genotypes that were abundant enough to test.

Seasonal Variation

Despite exhibiting low, rounded helmets, no evidence of hybridization was detected in the spring populations of Bantam Lake and Mt. Tom Pond (Table 3). The allelic arrays of these samples were nearly identical with those of the *Daphnia* with pointed helmets from the same lakes in midsummer. Although allele frequencies differed significantly between April and July samples in the two lakes (*PGM-2* and *PEPD* in Mt. Tom, and *PGM-2* in Bantam; G-tests of independence, $P < 0.005$ in each case), each allele fluctuation was less than 20%. Genotypic changes were also significant (G-tests of independence, $P < 0.005$ in each case) at these loci, but again, the genotypes that were prevalent in July were still prevalent in April.

Reinterpretation of Allozyme Variation

A summary of allozyme variation in prior studies is presented in Table 1. Data on alkaline phosphatase (Mort and Wolf 1985) and amylase (Stirling and McQueen 1987) variation were omitted because these loci were not examined in our study.

Lake St. George and Haynes Lake

As heterozygosity at *AO* and *PGM* is diagnostic of hybrids in the present study of Lake St. George, our allozyme reanalysis suggests that Stirling and McQueen's (1987) genotype 3 in Lake St. George and in Haynes Lake was an interspecific hybrid between *D. galeata mendotae* and *D. rosea*. Genotype 3 was also the only common multilocus genotype to possess exclusively

rounded helmets during the summer (Stirling and McQueen 1987). This summer phenotype is characteristic of hybrids but not of *D. galeata mendotae* (Taylor and Hebert 1992). The remaining genotypes (1, 2, 9, and 15) had diagnostic characters of pure *D. galeata mendotae*, as they were fast homozygotes at *AO* and possessed tall, pointed helmets in the summer.[1]

Lake Mitchell

Morphological and allozyme evidence in the present study suggests that *D. galeata mendotae* from earlier Lake Mitchell studies were actually *D. galeata mendotae* × *D. rosea*. The hybrids from the present study closely resemble the drawing of "*D. galeata mendotae*" from Lake Mitchell by DeMott (1981) and both possessed low, rounded helmets during midsummer. This was the only morph of "*D. galeata mendotae*" present in the lake during prior studies (W.R. DeMott, Indiana University – Purdue University, personal communication). In their Lake Mitchell allozyme study, Mort and Jacobs, (1981) detected 3 *GPI* genotypes (*ss*. *fs*, and *ff*) of *D. galeata mendotae*. The GPI^{fs} genotype prevailed in the lake with a frequency of 0.803. Although we had similar sample sizes to Mort and Jacobs (1981), we found only the heterozygous genotype (GPI^{ab}). As only two alleles at *GPI* have been detected in *D. galeata mendotae* from the eastern United States (this study; D. Taylor, unpublished data) with *D. rosea* monomorphic for the faster of these alleles, it is probable that the GPI^{fs} genotype of Mort and Jacobs (1981) is the same as the GPI^{ab} genotype in the present study.

GPI homozygotes may have become extinct since 1979. Yet the GPI^{ss} genotype is not likely to be the result of *D. galeata mendotae* × *D. rosea* because we have not detected the GPI^{s} allele in any *D. rosea* population. The possibility of the GPI^{ss} being created by $F_1 \times F_1$ also seems remote because the study of Mort and Jacobs (1981) occurred during the first summer of hybrid colonization. Moreover, the presence of only one multilocus hybrid genotype in our study is inconsistent with successful $F_1 \times F_1$ matings. Interestingly, the *GPI* homozygotes recorded for *D. galeata mendotae* by Mort and Jacobs (1981) electrophoretically match those of the other two *Daphnia* species in Lake Mitchell. In our samples, both *D. rosea* and *Daphnia catawba* Coker,1926 were present and *D. rosea* has a GPI^{ff} genotype whereas *D. catawba* has a GPI^{ss} genotype. As these *Daphnia* species and the *D. galeata mendotae* × *D. rosea* hybrids are strikingly similar in size and appearance, it is likely, then, that the uncommon genotypes present in the Mort and Jacobs (1981) study were actually *D. catawba* and *D. rosea*. The existence of homozygotes in Mort and Jacobs' (1981) data can be explained if misdiagnosis occurred 13.6% of the time (82/603). Fixed differences at other enzyme loci indicate that *D. catawba* do not hybridize with either *D. rosea* or *D. galeata mendotae* (D. Taylor, unpublished data).

Lake Morey

No evidence of hybridization was found in the genetic data for Lake Morey *D. galeata mendotae*. Our reevaluation of this population does, however, provide insight into Lake *Daphnia* population genetics. From 1981 to 1982, Mort and Wolf (1985) found genotypic frequencies at *sAAT* to be very stable in the lake. Assuming that the two alleles at *sAAT* in Mort and Wolf (1985) are the same two alleles that we found for *D. galeata mendotae*, then the genotypic frequencies are not significantly different 9 yr later ($G = 3.74$, df = 1, $P > 0.05$). Although *sAAT* variation showed continued stability, *GPI*, which was invariant in 1981, now showed two alleles with the less common allele having a frequency of 0.24.

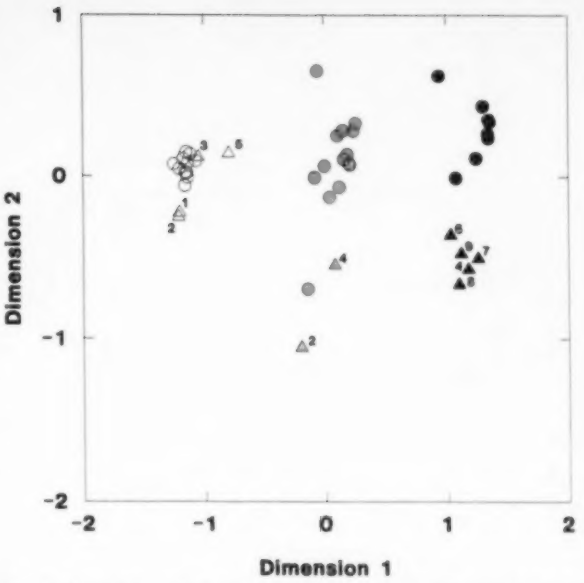

FIG. 3. Nonmetric multidimensional scaling of genetic distances (chord) derived from nine variable loci in the *D. galeata mendotae* complex ($R^2 = 0.993$). *PGM-1* was not included, as it was not available for all populations. Circles represent known populations of *D. galeata mendotae* (solid), *D. rosea* (open), and hybrids (hatched) from Indiana (Taylor and Hebert 1992). Triangles are filled in the same patterns and represent populations reexamined in this study. These are numbered as follows: 1, Guelph Pond, Ont.; 2, Lake Mitchell, Vt.; 3, Three-Lakes-Three, Mich.; 4, Lake St. George, Ont.; 5, Haynes Lake, Ont.; 6, Bantam Lake, Conn.; 7, East Twin Lake, Conn.; 8, Mt. Tom Pond, Conn.; 9, Lake Morey, Vt.

Within-Lake Spatial Distribution of Morphs

Malone and McQueen (1983) described two morphs of *D. galeata mendotae* from Lake St. George as possessing either a "very rounded head capsule" or a "head capsule produced into a point." Our reanalysis of Stirling and McQueen's (1987) data provides evidence that the polymorphism was created by hybrids coexisting in the lake with *D. galeata mendotae*. For the following reasons, it is probable that Malone and McQueen's (1983) rounded morph was also of hybrid origin whereas the pointed morph was true *D. galeata mendotae*. First, hybrids possess very rounded helmets at all times and Malone and McQueen (1983) sampled on October 11, a period when true *D. galeata mendotae* genotypes in Lake St. George rarely possess rounded helmets (Stirling and McQueen 1987). Second, hybrids (i.e., genotypes 3 and iii)) are dominant in the fall (Stirling and McQueen 1987).

Reexamination of Malone and McQueen's (1983) data provides evidence for vertical partitioning of hybrids and *D. galeata mendotae* in Lake St. George. They took 20 samples at each of four depths and recorded abundances of the two morphs of *D. galeata mendotae*. They concluded that the two morphs were acting as a "unispecies patch" because their abundances were

[1]Stirling and McQueen (1987) indicated that genotype 2 was heterozygous at *AO*, but it is clear from Stirling (1987) that this was a typographical error and that AO^{ff} is the original and correct assignment.

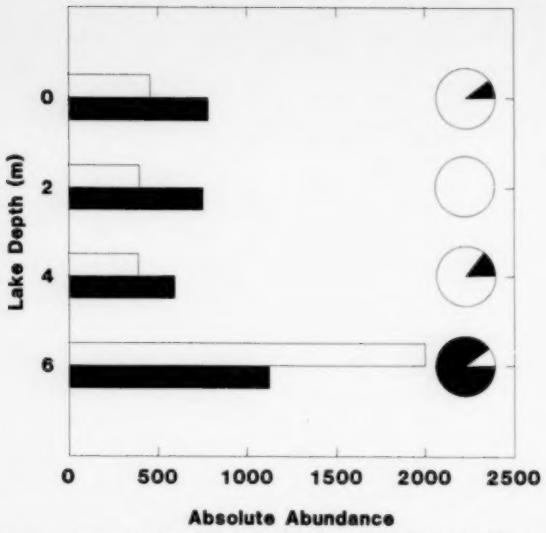

FIG. 4. Relationship between lake depth and abundance (in 500 L of water) of rounded-head (solid bars) and pointed-head (open bars) morphs in Lake St. George (data from Malone and McQueen 1983). Pie graphs indicate the proportion of 25-L samples (20 samples for each depth) across a horizontal grid in which a morph dominated.

correlated along horizontal sampling grids at three depths. Nevertheless, an examination of vertical distribution reveals heterogeneity of morph distributions (Fig. 4). The pointed-head morph (*D. galeata mendotae*) was significantly more abundant than the rounded-head morph (hybrids) at 10 cm, 2 m, and 4 m (Wilcoxon's signed ranks tests, $z = 3.57–3.92$, $P < 0.001$ for each depth). At 6 m, however, the rounded-head morph was more abundant than the pointed-head morph ($z = 2.95$, $P = 0.003$). The dominance relationship between morphs was consistent across the horizontal grid in the lake, as one morph prevailed in 85–100% of the samples for a given depth (Fig. 4).

Discussion

Our results show that the hybrid zone between *D. galeata mendotae* and *D. rosea* extends north into Ontario and east into Vermont. Thus far, southern Illinois marks the southern and western extremities of the zone (D. Taylor, unpublished data). Yet the entire hybrid zone is probably much larger because we have examined only a small portion of the region where *D. galeata mendotae* and *D. rosea* are sympatric. Possession of local parental alleles by hybrids further suggests that hybridization in this complex is due to local hybridization rather than dispersal of hybrids following their synthesis. Such widespread hybridization is rare in animals, as zones of sympatry between parent taxa are usually narrow (Harrison 1990).

Hybridization and Patterns of Morphological Variation

The existence of *D. galeata mendotae* × *D. rosea* hybrids accounts for among-lake spatial morphological variation in both Ontario and Vermont studies. During the midsummer, Haynes Lake (Ontario) and Lake Mitchell (Vermont) may contain only hybrids, while nearby lakes (Lake St. George and Lake Morey) are dominated by *D. galeata mendotae*. A spatial polymorphism results because hybrids possess rounded helmets, while *D. galeata mendotae* possess pointed or angulated helmets. Artificial polymorphism may also result when rounded-helmet *D. rosea* are identified as *D. galeata mendotae* (e.g., Three-Lakes-Three).

Interspecific hybrids also contribute to within-lake polymorphisms. The coexistence of low-, rounded-head hybrids with tall, pointed/angulated *D. galeata mendotae* in Lake St. George creates a dimorphic pattern under stratified conditions. Vertical partitioning of hybrids and *D. galeata mendotae* appears to occur and coincides with the metalimnion in Lake St. George (McQueen et al. 1989). Our reanalysis suggests that *D. galeata mendotae* dominate within the epilimnion, while hybrids dominate at greater depths. Brooks (1964) attributed such patterns to phenotypic plasticity and concluded that *D. galeata mendotae* develop helmets only when living in the warm epilimnion; individuals living below this layer were thought to lack helmets because of exposure to slow growth conditions.

Although we did not detect low-, rounded-helmet morphs in our reexamination of Brooks' (1964) study sites, there is evidence to suggest that his samples contained *D. galeata mendotae* × *D. rosea* hybrids. The juveniles depicted by Brooks (1964) in Queechy Lake possessed small anterodorsal denticles which are diagnostic of *D. galeata mendotae* × *D. rosea* hybrids in Indiana (Taylor and Hebert 1992). In addition, the drawing of a rounded-helmet adult from Brooks' (1964) study of Mt. Tom Pond is more similar to the hybrids from Lake St. George (in head and carapace shape, head width, and relative tail spine length) than to *D. galeata mendotae* that develop under low temperature conditions in Mt. Tom Pond (Fig. 2B). The low-, rounded-head morph in East Twin Lake was also probably of hybrid origin, as Brooks (1964) found the helmet dimorphism to be genetically controlled in this lake.

Hybridization can also distort patterns of seasonal polymorphism in *D. galeata mendotae*. Probable hybrid genotypes (Stirling and McQueen's iii and 3) increased in abundance relative to *D. galeata mendotae* from summer to winter in Lake St. George in both 1984 and 1985 (Stirling and McQueen 1987). The fall decay of helmets is exaggerated by treating hybrids and *D. galeata mendotae* as one taxon. The same abundance shifts occur in Indiana lakes where *D. galeata mendotae* coexist with hybrids (Taylor and Hebert 1992). The reanalysis of Bantam Lake and Mt. Tom Pond, however, reveals that hybrid–parent succession is not the sole explanation for seasonal polymorphism in *D. galeata mendotae*. The parent taxon is capable of expressing both the maximum and minimum helmets in these lakes. There was also no evidence of genotypic succession causing seasonal polymorphism from these lakes, as genotypic fluctuations were small.

Is there a genetic component to cyclomorphosis with *D. galeata mendotae*? Stirling and McQueen (1987) are the only investigators who have reported an association between allozyme and helmet phenotypes within a *Daphnia* species. We have shown, however, that this association was due to their inclusion of interspecific hybrids. Nevertheless, we did find a different association in Lake St. George, as the *PGM-2*^{bb} genotype was more often associated with a pointed-head shape than the *PGM-2*^{bd} genotype. The fast allele is the most common allele in *D. rosea* whereas the slow allele is the most common in *D. galeata mendotae* (Table 2). Such a pattern suggests that the *PGM-2*^{bd} genotype may, in some clones, mark the introgression of *D. rosea* alleles into *D. galeata mendotae* genomes. We have

found evidence that asymmetric introgression of this kind is widespread between *D. rosea* and *D. galeata mendotae* (Taylor and Hebert 1992; D. Taylor and P. Hebert, unpublished data). If helmet shape is under polygenic control, then introgression may affect helmet expression according to alien gene dosage. Thus, some *D. galeata mendotae* in Lake St. George may have attenuated helmets as a result of the introgression of *D. rosea* alleles for smaller, rounder helmets. Nevertheless, we found no association between introgressed alleles and cyclomorphosis in Bantam Lake and Mt. Tom Pond populations. Consequently, our results support earlier investigations in concluding that the phenomenon of cyclomorphosis in pure *D. galeata mendotae* is due to phenotypic plasticity while acknowledging that different genotypes may have different norms of reaction (Jacobs 1961; Mort 1989).

Dynamics of Hybridization

Because the GPI^{s} allele is rare in *D. galeata mendotae* and absent in *D. rosea* (Table 2), the finding of the same dominant hybrid genotype GPI^{ss} in Lake Mitchell in both 1979 and 1990 suggests that hybrid clones can persist from year to year. Certainly, hybrid genotypes in Lake St. George and Haynes Lake overwinter as free-swimming *Daphnia*. The observation of low clonal diversity in both of these hybrid populations as well as their potential to overwinter may indicate that dominant clones are not only persistent but also may arise from only a few hybridization events (Brooks 1957). Unfortunately the contribution of selection to these observed patterns is unknown.

Of course, a hybrid clone that can neither disperse nor respond rapidly to environmental change has a rather limited evolutionary potential. We have provided evidence that hybrid clones once dominated both Queechy Lake and Haynes Lake but have since become rare or extinct. Yet such hybrid clones are not necessarily the "evolutionary dead-ends" that Fryer (1991) suggested. Haynes Lake now contains *D. rosea* with alleles that are typical of *D. galeata mendotae*. Thus introgression may be the evolutionary legacy of hybrids that act as conduits for gene flow between *D. galeata mendotae* and *D. rosea*.

Reevaluation of *D. galeata mendotae* Ecology

If our interpretation of Mort and Jacobs' (1981) data is correct, then their manipulation experiments indicated ecological differences between *D. rosea* and the interspecific hybrid *D. galeata mendotae* rather than among genotypes of *D. galeata*. Mort and Jacobs (1981) reported a relative increase in the GPI^{fs} genotype over GPI^{ff} after increasing density either directly or by adding nutrients (C, N, P). Our reinterpretation suggests that "*ff*" genotypes represented *D. rosea* and "*fs*" genotypes represented hybrids. Therefore the experiments provided evidence that hybrids are more fit than *D. rosea* under these conditions. Mort and Jacobs (1981) suggested that the increased fitness was due to heterosis at the *GPI* locus, but this relationship is untenable if the GPI^{fs} genotype marked an entire hybrid genome. It is worth noting that Mort et al. (1989) found no genotypic responses at *GPI* to density during similar manipulations with European *Daphnia galeata galeata*. There was, however, a relative increase in *D. galeata mendotae* × *Daphnia hyalina* hybrids compared with the parent taxa (see Mort 1990), producing results that parallel our reinterpretation of Mort and Jacobs' (1981) experiments. It should also be noted that only hybrid genotypes were detected on two of three sampling dates in the enclosure station of Stirling and McQueen (1987).

Mort (1986) also performed a series of experiments with the invertebrate predator *Chaoborus* to gain insight on the adaptive significance and maintenance of helmet polymorphism in *D. galeata mendotae*. *Daphnia galeata mendotae* from Lake Morey were used to represent the pointed-helmet phenotype and *D. galeata mendotae* from Lake Mitchell were used to represent the low-helmeted phenotype. The experiments revealed that high-helmeted *D. galeata mendotae* from Lake Morey was more difficult for *Chaoborus* to capture and ingest than the low-helmeted morph of Lake Mitchell. Our study reveals that the low-helmeted morphs of *D. galeata mendotae* were probably F_1 hybrids. Therefore the data actually provided evidence that hybrids are more susceptible than *D. galeata mendotae* (with pointed helmets) to *Chaoborus* predation. Invertebrate predation might then be an important factor in determining the success of interspecific hybrids.

Taylor and Hebert (1992) found that *D. rosea* were associated with lakes that are smaller in surface area than lakes associated with *D. galeata mendotae*. In this present study, *D. rosea* also occurred in the small water bodies (Guelph pond, Three-Lakes-Three, Haynes Lake, Lake Mitchell), while *D. galeata mendotae* tended to occur in large lakes (Lake St. George being the exception). This pattern of neighbouring sympatry is obviously due to ecological rather than dispersal limitations, as habitat associations exist across broad areas of North America that both species have colonized since deglaciation. Moreover, our finding of widespread local hybridization between these species indicates that populations of each species are often within dispersal range of one another.

The exact nature of the ecological forces that segregate *D. galeata mendotae* and *D. rosea* remain to be addressed. Yet, as species replacements have occurred within the same lake (D. Taylor, unpublished data), we agree with Brooks (1957) that habitat associations based on size of water bodies are most probably due to food availability, competition, and predation rather than to abiotic factors. These biotic forces are presumably responsible for differences in species composition between the adjacent small, hardwater kettle lakes, Haynes Lake and Lake St. George. *Daphnia rosea* are prevalent in Haynes Lake but absent in Lake St. George, whereas *D. galeata mendotae* are prevalent in Lake St. George but absent in Haynes Lake (Stirling and McQueen 1987; this study). *Daphnia* in Lake George are exposed to greater planktivore pressure (from yellow perch (*Perca flavescens*) and golden shiner (*Notemigonus crysoleucas*)) and more eutrophic conditions than *Daphnia* in Haynes Lake (Mazumder et al. 1990; Lafontaine and McQueen 1991). If this ecological pattern were widespread, it might be concluded that intense planktivory prohibits *D. rosea* from becoming established in *D. galeata mendotae* dominated lakes, while intense competition prevents *D. galeata mendotae* from becoming established in *D. rosea* dominated lakes. Indeed, studies from across North America (where taxonomy is not in question) do suggest that *D. rosea* are a strong exploitative competitor in lakes where vertebrate predation is negligible (Clark and Carter 1974; DeMott 1983; Neill 1985). The success of *D. galeata mendotae*, on the other hand, has been associated with intense vertebrate predation, and it is often replaced by other *Daphnia* species when predators (e.g., yellow perch, cisco (*Coregonus artedi*), and golden shiner) become scarce (Zaret and Suffern 1976; Mills and Forney 1988; Rudstam et al. 1993).

Hybrids between *D. galeata mendotae* and *D. rosea* have been detected only in lakes where one or both parent species are absent

or rare. Because the hybrids are locally produced and often more abundant than coexisting parent taxa, hybrid clones must be fitter than one or both parents under certain environmental conditions. Investigations of hybridization among Eurasian lake *Daphnia* suggest that a combination of differential use of the environment (temporal and spatial) and hybrid vigor contributes to their success (Wolf and Mort 1986; Wolf 1987; Mort 1990; Weider and Wolf 1991). Likewise, our reevaluation suggests that some *D. galeata mendotae* × *D. rosea* clones respond differently than parent taxa with respect to vertical segregation, invertebrate predation, and nutrient enrichment. As these phenomena involved only a few hybrid clones, however, it is unclear whether such patterns represent general hybrid features or unique features of these clones that arose as a consequence of selection.

The ecological conditions that separate *D. galeata mendotae* and *D. rosea* and that lead to hybrid success need to be investigated by multilake studies. Although these two species have been prominent in past limnological studies, taxonomic confusion reduces their value for comparative biology. Nevertheless, we have shown that when genetic and morphological information is available, successful reinterpretation of existing studies is possible. We therefore recommend that investigators genetically compare individuals from their study sites and individuals of a known taxon. This study reveals that such a comparison is especially important in lakes where rounded-helmet *D. galeata mendotae* occur in summer months and where *D. galeata mendotae* are thought to coexist with *D. rosea*.

Acknowledgments

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Reproductive Cycle and Fecundity of Primiparous and Multiparous Female Snow Crab, *Chionoecetes opilio*, in the Northwest Gulf of Saint Lawrence

Bernard Sainte-Marie

Ministère des Pêches et des Océans, Direction des sciences des pêches, Institut Maurice-Lamontagne, 850 route de la Mer, C.P. 1000, Mont-Joli, QC G5H 3Z4, Canada

Sainte-Marie, B. 1993. Reproductive cycle and fecundity of primiparous and multiparous female snow crab, *Chionoecetes opilio*, in the northwest Gulf of Saint Lawrence. Can. J. Fish. Aquat. Sci. 50: 2147–2156.

A total of 1 691 mature female *Chionoecetes opilio* was collected by beam trawl in Baie Sainte-Marguerite on seven occasions from April 1991 to May 1992. Quantitative analyses of ovaries, brood, eggs, and spermathecal contents were performed on a subsample of 318 females. Females moulted to maturity in March and April. Development of ovaries and brood were phased and lasted 24–27 mo. Eggs hatched mainly from April to June. Spermathecae contained zero to three ejaculates, and the mean blotted weight of freshly deposited ejaculates was significantly greater in primiparous (0.513 g) than in multiparous (0.249 g) females. The weight of stored ejaculates decreased over time independently of spawning events. Fecundity was correlated positively with carapace width and negatively with mean egg diameter. Eggs of primiparous females were on average 1.4–2.7% larger, but 16.4–22.7% fewer per brood, than those of multiparous females. Some large multiparous females had small broods, due possibly to lesser fertility. Females probably hatch only two broods in a lifetime and produce a total of 81 630 to 83 143 larvae at 57.4 mm carapace width. Primiparous females apparently contribute >40% of larvae produced by the population.

Un total de 1 691 femelles matures de *Chionoecetes opilio* a été capturé à l'aide d'un chalut à perche au cours de sept campagnes effectuées dans la baie Sainte-Marguerite d'avril 1991 à mai 1992. L'analyse quantitative des ovaires, de la portée, des oeufs et du contenu des spermathèques a été réalisée sur un sous-échantillon de 318 femelles. Les femelles deviennent matures en mars et avril. Les ovaires et la portée se développent de façon synchrone sur une période de 24 à 27 mo. L'éclosion des oeufs a lieu surtout d'avril à juin. Les spermathèques contiennent de zero à trois éjaculats et le poids humide moyen des éjaculats récemment déposés est significativement plus élevé chez les femelles primipares (0,513 g) que chez les femelles multipares (0,249 g). Le poids des éjaculats entreposés décroit avec le temps et ce, indépendamment de la ponte. La fécondité était corrélée positivement avec la largeur de la carapace et négativement avec le diamètre moyen des oeufs. Les oeufs des femelles primipares sont 1,4 à 2,7 % plus gros, mais 16,4 à 22,7 % moins nombreux par portée, que ceux des femelles multipares. Quelques femelles multipares de grande taille ont de petites portées, possiblement pour cause d'infertilité partielle. Les femelles ne libèrent probablement que deux portées au cours de leur vie et une femelle de 57,4 mm de largeur de carapace produirait au total entre 81 630 et 83 143 larves. Les femelles primipares produiraient >40 % des larves de la population.

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Females of the genus *Chionoecetes* (Brachyura: Majidae) have paired spermathecae and are iteroparous. Four types of females may be recognized (Sainte-Marie and Hazel 1992). Immature and pubescent females have narrow abdomens indicating a nonreproductive state. Pubescent females, however, have ripe ovaries and are committed to maturity at the next moult. Mature females are characterized by a broad abdomen and include primary (primiparous) and repeat (multiparous) spawners.

Pubescent females moult to maturity, generally with the help of a male (Watson 1970, 1972; Donaldson and Adams 1989; Sainte-Marie and Hazel 1992), and must be fertilized within days in order to produce a viable brood (Paul et al. 1983; Paul and Adams 1984). The moult to maturity is considered to be the last (Yoshida 1941; Ito 1963; Watson 1972; Hilsinger 1976), so females subsequently mate in the hard-shell condition (Somerton 1981; Adams 1982; Taylor et al. 1985). However, like other majids, female *Chionoecetes* may produce more than one viable brood from sperm deposited in the spermathecae (e.g., Hartnoll 1965; 1969; Watson 1970, 1972; Adams and Paul 1983; Paul et al. 1983; Paul 1984; Diesel 1989).

Primiparous and multiparous *Chionoecetes* may differ in fecundity and in quality of progeny. Development of ovaries in brachyuran crabs is limited by the volume of the body cavity (Hines 1982, 1992). In Tanner crab, *C. bairdi*, primiparous females are less fecund than multiparous females for a constant carapace width, possibly because brood size of primiparous females depends on premoult body size (Somerton and Meyers 1983). However, pubescent females must also allocate energy simultaneously to somatic and gonadic growth, with unknown effects on broods (Somerton and Meyers 1983; Hartnoll 1985; Elner and Beninger 1992). Finally, for *C. bairdi* and snow crab, *C. opilio*, there is evidence that mates of pubescent females are smaller than those of multiparous females (Somerton 1982;

TABLE 1. Criteria used for rating exoskeleton condition, apparent age, and brood development of mature female *C. opilio*.

Female characteristic	Criteria
Exoskeleton condition	
Clean soft	Exoskeleton soft and immaculate; carapace devoid of epizoites; chelae iridescent
Clean hard	Exoskeleton hard and immaculate; carapace devoid of epizoites; chelae iridescent
Intermediate	Exoskeleton hard and marked or lightly scarred; carapace with small epizoites; chelae more or less iridescent
Dirty hard	Exoskeleton hard, dirty, and heavily scarred; carapace with extensive bryozoan mats and other numerous and often large epizoites; chelae dull
Dirty soft	Exoskeleton soft, dirty, and heavily scarred; carapace with extensive bryozoan mats and other numerous, large, and often dead epizoites; chelae dirty
Apparent age	
New recruit	Exoskeleton in clean soft, clean hard, or intermediate condition; no mating scars on pereiopods
Old recruit	Exoskeleton in intermediate, dirty hard, or dirty soft condition; generally with mating scars on pereiopods
Brood development	
Stage 1	No brood
Stage 2	Orange eggs
Stage 3	Dark-orange eggs with developing eyespots
Stage 4	Dark-brown or purple, eyed eggs
Stage 5	Empty egg shells, possibly mixed with dark, eyed eggs
Stage 6	Degenerating eggs

Sainte-Marie and Hazel 1992; Stevens et al. 1993). The vast majority of males that mate with both types of females are morphometrically mature (Conan and Comeau 1986; Ennis et al. 1988, 1990; Sainte-Marie and Hazel 1992; Stevens et al. 1993) and the size at which this condition is achieved might be an inheritable trait (Conan 1986; Elner et al. 1986; Bailey and Elner 1989). Consequently, primiparous and multiparous females may produce genetically different progenies.

Commercial exploitation could lead to increased participation of small males in reproduction. Male competition for females and size dimorphism in favour of males in mating pairs are common features of breeding Brachyura (Christy 1987), and specifically of *Chionoecetes* (Brown and Powell 1972; Taylor et al. 1985; Conan and Comeau 1986; Ennis et al. 1988, 1990; Sainte-Marie and Hazel 1992). *Chionoecetes* fisheries in North America target only the largest males because of a minimum size regulation that excludes all females and small males (Miller 1976). A possible consequence is a reduction of competition and increase of the breeding activity of small males, as suggested for *Chionoecetes* (Bailey and Elner 1989) and other brachyurans (Butler 1960). Alternatively, if suitable male mates are unavailable, large primary spawners may extrude and carry nonviable broods, while large repeat spawners may rely on stored sperm acquired from small males at the first mating.

In the present work, I compared the reproductive cycle and fecundity of primiparous and multiparous *C. opilio* in light of the above hypotheses. A prime objective was to determine the relative contribution of primiparous and multiparous females to population reproductive output. This study also serves as a backdrop for experiments on reproduction and heritability in *C. opilio*.

Materials and Methods

Collection and Treatment of Females

Snow crab were collected from Baie Sainte-Marguerite (~50°06′N, 66°35′W), in the northwest Gulf of Saint Lawrence (for maps and site description, see Sainte-Marie et al. 1988; Sainte-Marie and Hazel 1992). The Bay is intensively fished and despite an area of only ~270 km^2 shoreward of the 150-m isobath, it yielded ~218 t of snow crab in 1992, which represented the highest catches per unit area on the north shore of the Gulf.

Sampling was conducted on April 11–15, June 19–24, August 22–29, October 23–29, and December 11–12, 1991, and on March 19–21 and May 12–14, 1992. Snow crab were collected with a 3-m beam trawl during 15- to 20-min tows at three randomly selected locations in each of three depth strata: 4 to ≤20 m, >20 to ≤80 m, and >80 to 140 m. Ship speed was 2.5–3 kn. Trawl contents were sorted onboard. Mature females were recognized by their large and rounded abdomen which almost completely covers the cephalothoracic sternites. These females were measured and their exoskeleton condition, apparent age, and brood development determined as described below and in Table 1.

On the first six dates, a sample of mature females was fixed by injection of, and immersion in, 4% buffered formalin in seawater. In the laboratory, preserved females were again rated for exoskeleton condition, apparent age, and brood development according to criteria in Table 1. The widths of carapace (CW) and fifth abdominal segment (AW) at their broadest points were measured with a vernier caliper to the nearest 0.01 mm. The numbers of missing and of regenerated pereiopods were recorded.

The weight of blotted females was determined to the nearest 0.01 g. Weights of blotted brood and ovaries, along with two subsamples each of 20 eggs, were measured to the nearest 10^{-5} g. Fecundity was determined as the ratio of brood weight to mean egg weight. Diameters of 10 randomly chosen eggs from each female were measured to the nearest 0.01 mm with an eyepiece micrometer. When the egg was ovoid, as occasionally was the case, diameter was calculated as the mean of the long and short axes. The spermathecae were sectioned longitudinally and the discretely layered ejaculates were counted. Recent ejaculate was whiter than old ejaculate, as reported for *C. bairdi* (Adams and Paul 1983), and was located in the ventral part of the spermatheca. The weight of blotted recent ejaculates from both spermathecae was determined to the nearest 10^{-5} g.

Analysis of Data

Data were $\log_{10}$ transformed to account for the known allometric relationships between reproductive traits (e.g., Hines 1982). The resulting multivariate data set, exclusive of ejaculate weight, was explored using partial correlation analysis to measure the degree of association between two variables while the effects of other specified variables were held constant (Sokal and Rohlf 1981). Simple or multiple linear regression was used to describe relationships between variables. Comparison of significant regressions proceeded in two steps. First, the slopes were tested for equality by analysis of variance. Second, when slopes were equal, elevations were tested for equality by analysis of covariance (Sokal and Rolf 1981). Statistics used for univariate data are the mean and standard error.

Results

Seasonal Changes in Exoskeleton Condition and Brood

A total of 1691 mature females, ranging from 40 to 77 mm CW, were sampled from March 1991 to May 1992. Clean soft females were captured only in April 1991 and in March 1992 in shallow waters, coincident with diver observations of moulting pubescent females on bottoms <35 m deep (Sainte-Marie and Hazel 1992, and unpublished data). The 1991 new recruits were distinguished from other females with a high degree of confidence until December 1991. Thereafter, accumulated fouling and scarring rendered categorization uncertain. For the March 1992 sample, a conservative approach was adopted and only the least fouled and least scarred females with hard exoskeletons were categorized as 1991 new recruits. Thus, I was able to distinguish new from old recruits for 9 mo after the moult to maturity; Ito (1967) separated these two types of females for 6 mo by shell hardness alone.

Females with Stage 2 broods (orange eggs) dominated samples at all times of the year, but there was obvious seasonal variation (Fig. 1). All but one female had Stage 2 broods in August 1991. Thereafter, the proportion of females with Stage 3 and Stage 4 broods increased gradually to a maximum of 24.3% in March 1992, similar to the observed 22.7% in April 1991. Females with Stage 5 (hatching) broods were found most frequently in June 1991 and in May 1992. Barren females were very scarce throughout the year.

Ovary and Brood Development

None of the 318 mature females dissected in the laboratory had a second carapace, nor incidentally did any of the 1691 females from field samples have a visible second shell. These features normally characterize individuals that are committed to moulting (Sainte-Marie and Hazel 1992).

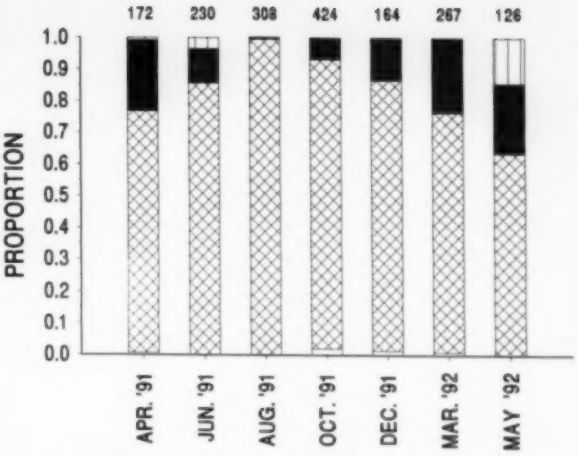

FIG. 1. Proportions of female *C. opilio* with broods at different stages of development in Baie Sainte-Marguerite from April 1991 to May 1992. Sample size appears above the bars. The white part of the bars represents barren females, cross-hatching represents females with Stage 2 broods (orange eggs), black represents females with Stage 3 and 4 broods (eyed eggs), and vertical hatching represents females with Stage 5 broods (empty egg shells present).

Females represented in scattergrams of weight of ovaries on carapace width were separated into groups with different spawning histories, on the basis of apparent age and brood development (Fig. 2). In each scattergram, an oblique line was traced to separate 1991 new recruits from old recruits higher on the ordinate. Another oblique line in the March scattergram separated 1992 new recruits from 1991 new recruits. In the April, June, and August scattergrams, an additional oblique line separated females with Stage 3–5 broods from those with Stage 2 broods. The groups formed by these lines were numbered I–IV. All lines had a slope of 2.9, which was determined by trial and error to be the only slope that consistently allowed separation of females according to the above criteria. Work by B. Sainte-Marie and G.A. Lovrich (in preparation) provided a posteriori justification for the slope value and parallelism of separating lines. First, the 2.9 slope value fell within the 95% confidence interval of the regression coefficient of weight of ovaries on carapace width determined for 84 primiparous females sacrificed in formalin ≤12 h after extrusion of eggs. Second, at various fixed times before or after brood extrusion by primiparous females, the slopes of regressions of weight of ovaries on carapace width were found to be constant, while ordinates (*y*-intercepts) increased with ovary (or brood) development.

The weight of ovaries of 1991 new recruits increased from April 1991 to March 1992 but broods remained in Stage 2. In June 1991, the presence of females with Stage 5 (hatching) broods and an influx of many old recruits to Group II indicated that larval release and egg extrusion were underway for Group IV females. The 1991 recruits thus served as "tracers" for old recruits that respawned in 1991. Weight of ovaries in Group III also increased from April 1991 to March 1992, while broods remained in Stage 2 until August 1991 and from thereon

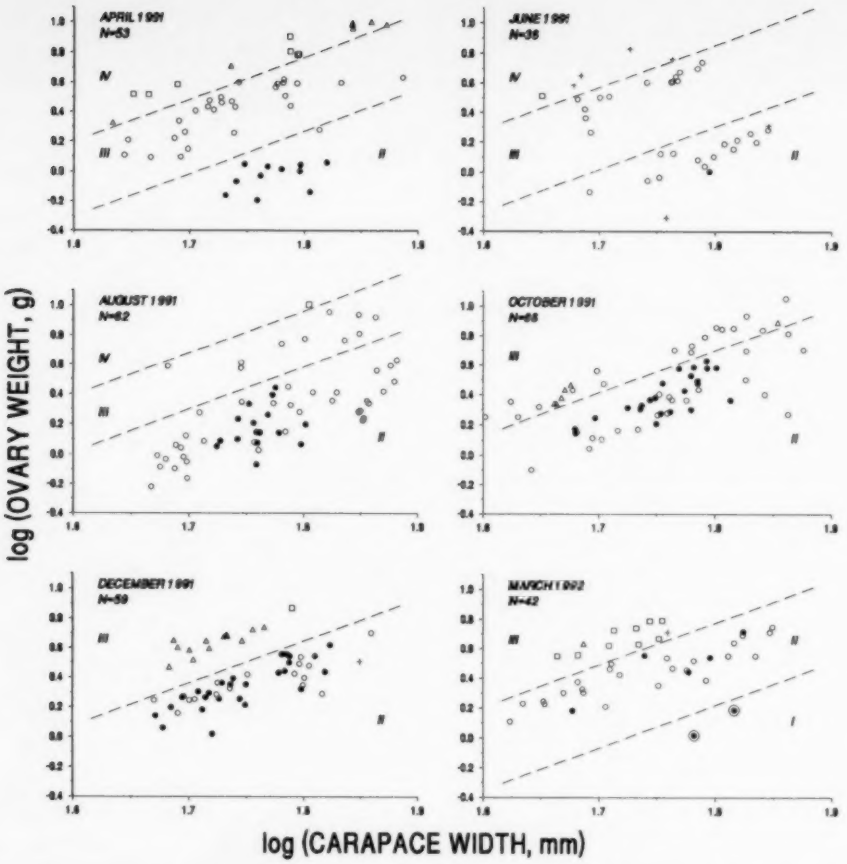

FIG. 2. Scattergrams of weight of ovaries on carapace width for mature female *C. opilio* collected in Baie Sainte-Marguerite from April 1991 to March 1992. Broken lines were fitted by eye and all have the same slope; they separate female Groups I–IV on the basis of apparent age and brood development. Group II females in April 1991 and Group I females in March 1992 had clean soft exoskeletons. Note that the broken line separating Group IV from Group III in April 1991 is in exactly the same position as the line separating Group III from Group II in March 1992. ⊙; 1992 new recruit with Stage 2 brood (orange eggs); ●, 1991 new recruit with Stage 2 brood; ○, old recruit with Stage 2 brood (orange eggs); △, old recruit with Stage 3 brood (eyed eggs); □, old recruit with Stage 4 brood (dark, eyed eggs); +, old recruit with Stage 5 (hatching) or no brood; *N*, sample size.

gradually changed to Stage 4.

Group II females marked by plus signs in June and December scattergrams were barren or had degenerating broods. One of these females (1.76 log CW, June) was senescent, judging from the dirty soft exoskeleton and necrosing gills. The scarcity of 1991 new recruits in June may be an artefact of sampling: in May and June, females moving offshore away from the shallow moulting ground (Sainte-Marie and Hazel 1992) cross relatively steep terrain which is not adequately sampled by our beam trawl.

The slopes of regressions of weight of ovaries on carapace width did not vary across female groups and sampling times ($P = 0.816$, June Group IV and March Group I excluded), but analysis of covariance pointed to significant differences in elevation ($P < 0.001$). For each combination of female group and sampling period, the weight of ovaries was adjusted (Fig. 3) to the overall mean carapace width of females (57.4 mm). Adjusted weight of ovaries increased between April 1991 and March 1992 from –0.065 (i.e., 0.86 g) to 0.470 (i.e., 2.95 g) in Group II and from 0.458 (i.e., 2.87 g) to 0.762 (i.e., 5.78 g) in Group III. Adjusted weight of ovaries did not differ between Group I in March 1992 and Group II in April 1991 ($P = 0.434$), between Group II in March 1992 and Group III in April 1991 ($P = 0.636$), and between Group III in March 1992 and Group IV in April 1991 ($P = 0.483$). Adjusted weight of ovaries in Group IV varied from 0.735 (i.e., 5.44 g) in April 1991 to 0.875 (i.e., 7.51 g) in August 1991, but differences for any pair of sampling dates (April, June, or August 1991) were not significant ($P \geq 0.125$). Incidentally, the log weight of ovaries ≤12 h after extrusion of eggs was estimated by regression to be -0.08 ± 0.07 for a 57.4 mm CW primiparous female sacrificed in formalin (B. Sainte-Marie and G.A. Lovrich, in preparation), a value very close to the -0.06 ± 0.03 obtained for Group II females in March 1991 (Fig. 2). This confirms that new recruits sampled in March 1991 were beginning their reproductive cycle.

The weight of ripe ovaries of females in Group IV was comparable with the weight of recently extruded broods in old

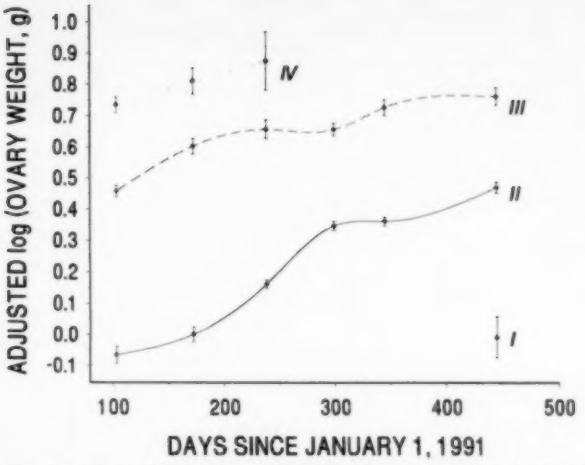

FIG. 3. Adjusted log weight ± 1 SE of ovaries as a function of elapsed time for Groups I–IV of mature female *C. opilio* collected in Baie Sainte-Marguerite from April 1991 to March 1992. Weight of ovaries was standardized for a constant 57.4 mm CW by analysis of covariance.

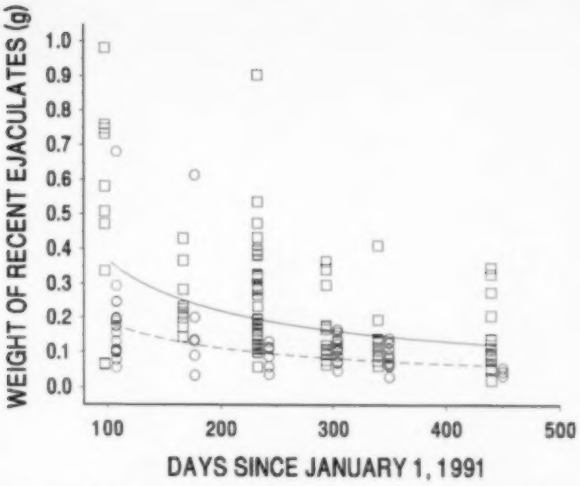

FIG. 4. Weight of recent ejaculates in spermathecae as a function of elapsed time for Groups II and III of mature female *C. opilio* collected in Baie Sainte-Marguerite from April 1991 to March 1992. Squares and solid line ($Y = 11.455 \cdot X^{-0.745}$) represent Group II females; circles and broken line ($Y = 5.794 \cdot X^{-0.745}$) represent Group III females. Adjusted equations were fitted to the data after equality of slopes was demonstrated. Data points are staggered by −10 d (□) and +10 d (○) about sampling time.

recruits from Group II. The predictive regression of brood weight (BW, in grams) on carapace width (CW, in millimetres) for the June 1991 sample of Group II old recruits was

$$(1)\quad \log BW = 1.967 \log CW - 2.513$$

$$N = 14,\ r^2 = 0.714,\ F = 27.41,\ P < 0.001.$$

From this regression, the weight of a recently extruded brood estimated for a 57.4 mm CW female was 0.948 ± 0.055 (i.e., 8.87 g), a value close to the adjusted weight of ovaries of Group IV (i.e., ripe) females in June (0.811 ± 0.042) and August (0.875 ± 0.094) of 1991.

Female maturity indices used so far with *Chionoecetes* species were based on the ratio of weight of ovaries to body weight exclusive of ovaries and, sometimes, of brood. These indices are biased if the female is not whole (Hilsinger 1976). For my sample of females, regression of total body weight (FW, in grams) on carapace width (CW, in millimetres) and on numbers of missing (MP) and of regenerated (RP) pereiopods gave

$$(2)\quad \log FW = 2.773 \log CW - 0.023\ MP - 0.022\ RP - 3.020$$

$$N = 318,\ R^2 = 0.939,\ F = 1622.79,\ P < 0.001.$$

The three coefficients were highly significant ($P < 0.001$). Each pereiopod thus accounted for about 5.1% of total body weight. Since only 55% of the females were whole, with the remaining females each missing up to five pereiopods or having up to two regenerated pereiopods, weight of ovaries adjusted for carapace width is a more appropriate index of maturity than indices based on weight ratios. Another advantage is that carapace width does not change after the moult, while body weight exclusive of ovaries and brood is subject to temporal variation due to somatic growth and fouling.

Number and Weight of Ejaculates

Females judged to be new recruits ($N = 84$) had zero (1.2% of 84) or one (98.8%) ejaculate per spermatheca, while females judged to be old recruits ($N = 234$) had one (10.3%), two (88.0%), or three (1.7%) ejaculates per spermatheca. In four females, one spermatheca contained an ejaculate, while the other was empty. The weight of recent ejaculates was not correlated with female carapace width ($r = 0.073$, $P > 0.33$).

The total quantity of ejaculate remaining in spermathecae, shortly after brood extrusion, was greater in new than in old recruits. Twelve of 13 new recruits from April 1991 and March 1992, compared with 10 of 15 old recruits in Group II from April and June 1991, had recent ejaculates in their spermathecae. The weight of recent ejaculates was 0.513 ± 0.079 g in new recruits and 0.249 ± 0.028 g in old recruits (t-test: $t = 2.91$, $P = 0.009$). Up to 0.982 g of recent ejaculate was found in one female (Fig. 4), a primary spawner sampled in April 1991.

Recent ejaculates in the spermathecae gradually darkened and decreased in weight over time ($P < 0.001$) in Group II and Group III females (Fig. 4). Slopes of regressions of ejaculate weight on time were similar for both groups of females ($F = 1.79$, $P = 0.183$), but elevations differed significantly ($F = 49.44$, $P < 0.001$). Adjusted ejaculate weight was −0.705 ± 0.026 (i.e., 0.197 g) in Group II females and −1.001 ± 0.035 (i.e., 0.099 g) in Group III females. The presence of large quantities (>0.3 g) of recent ejaculate in the spermathecae of a few ovigerous females in Groups III (Fig. 4) and IV (not shown) indicates that females may occasionally be inseminated during the spring breeding period, prior to hatching of their eggs.

Differences in Fecundity and Egg Diameter

Some females were excluded from analyses of fecundity and egg diameter (Fig. 5) because they were barren ($N = 6$), had begun to release their larvae ($N = 2$), or had unusually small

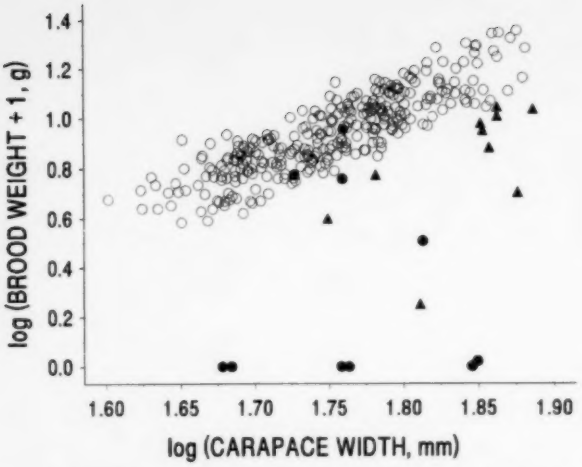

FIG. 5. Scattergram of brood weight on carapace width for 318 female *C. opilio* collected in Baie Sainte-Marguerite from April 1991 to March 1992. ○, female considered in analyses of brood weight, fecundity, and egg size; ●, female with hatching, degenerating, or no brood; ▲, female with normal-looking but undersized brood.

broods ($N = 12$). In the last category, two females had degenerating Stage 2 broods; one was a 1991 new recruit with empty spermathecae. Ten other females, including seven old recruits from Group II representing 36.8% of all females ≥71 mm CW, had undersized but otherwise apparently normal broods (Fig. 5).

Partial correlations between weight of ovaries, carapace width, abdomen width, egg diameter, and brood weight or fecundity appear in Table 2. Two separate analyses were performed because fecundity was not measured independently of brood weight. Besides the obvious correlation between widths of carapace and abdomen, five strong partial correlations were of interest. Brood weight and fecundity were positively correlated with carapace width, egg diameter was negatively correlated with fecundity, and brood weight and egg diameter were positively correlated with weight of ovaries, Thus, although there was no change in the appearance of eggs in Group II females with time (Fig. 2), individual eggs increased in volume as ovaries increased in weight.

Two different approaches were taken to contrast fecundity and egg diameter between primiparous and multiparous females. In the first, females with one ejaculate in at least one spermatheca were considered to be primiparous, while females with two or more ejaculates in at least one spermatheca were considered to be multiparous. In the second approach, new and old recruits in Group II from the April–December 1991 period were considered to be primiparous and multiparous, respectively. Both approaches led to the same conclusions, but the definition based on apparent age always produced greater contrasts between reproductive traits of primiparous and multiparous females. The relative differences in brood weight, fecundity, and egg diameter between primiparous and multiparous females are presented below as ranges that reflect the two approaches.

There was no significant difference between the slopes of regressions of log body weight and of log brood weight on log carapace width for whole (i.e., no missing or regenerated pereiopods) primiparous and multiparous females ($P > 0.15$). This was expected, since brood weight scales isometrically to body weight in a variety of other brachyuran crabs (Hines 1982). Elevations of regressions differed significantly ($P < 0.001$). Using values of body weight and brood weight adjusted for a constant carapace width by analysis of covariance, I calculated that brood weight averaged 10.0–10.8 and 11.8–11.9% of body weight in primiparous and multiparous females, respectively.

TABLE 2. Partial correlations between weight of ovaries (OW), carapace width (CW), abdomen width (AW), egg diameter (ED), and brood weight (BW) above diagonal or fecundity (FEC) below diagonal for 298 female *C. opilio* collected in Baie Sainte-Marguerite from April 1991 to March 1992. Note underlined coefficients ($P < 0.01$). All data were log transformed.

	OW	CW	AW	FEC\BW	ED
OW	—	0.08	−0.09	<u>0.29</u>	<u>0.27</u>
CW	0.12	—	<u>0.92</u>	<u>0.26</u>	−0.07
AW	−0.08	<u>0.94</u>	—	0.06	0.03
FEC\BW	0.17	<u>0.22</u>	0.03	—	0.08
ED	<u>0.35</u>	0.00	0.04	<u>−0.23</u>	—

Multiple regression performed separately on Group II and Group III females showed that fecundity varied with carapace width but was independent of weight of ovaries, as expected from Table 2. Because weight of ovaries increased with time since extrusion (Fig. 3), this observation strongly suggested that fecundity did not change over time. This conclusion was confirmed for Group II and Group III females by multiple regression of log fecundity on log carapace width and on log time elapsed since January 1, 1991. In both cases, fecundity varied positively with carapace width ($P < 0.001$) but independently of elapsed time ($P > 0.390$). Therefore, further comparisons of fecundity did not take into account the temporal component.

Overall predictive regressions of fecundity on carapace width appear in Table 3. The fecundity estimates for primiparous and multiparous females of 57.4 mm CW were, respectively, 37 870 and 45 273 eggs when female type was defined by number of ejaculates and, respectively, 35 597 and 46 033 eggs when female type was defined by apparent age. Thus, primiparous females were about 77.3–83.6% as fecund as multiparous females.

Egg diameter increased with time (see above), from a minimum of 0.58 mm for recently extruded, bright-orange eggs to a maximum of 0.83 mm for ripe, dark-purple eggs. Additionally, multiple regression performed separately on Group II and Group III females showed that log egg diameter varied positively with log time elapsed since January 1, 1991 ($P < 0.006$), and independently of log carapace width ($P > 0.05$). Slopes of regressions of log egg diameter on log weight of ovaries were not significantly different ($P > 0.25$) between primiparous and multiparous females, but elevations differed significantly ($P < 0.015$). For a constant weight of ovaries, egg diameter of primiparous females was 1.4–2.7% greater than that of multiparous females. Incidentally, using the weight of ovaries in lieu of elapsed time can account for variability in the timing of individual reproductive cycles.

Discussion

Reproductive Cycle

Several lines of evidence support the conclusion that females of *C. opilio* in Baie Sainte-Marguerite have a biennial repro-

TABLE 3. Mature female *C. opilio* from Baie Sainte-Marguerite. Regressions of fecundity (FEC, number of eggs per brood) on carapace width (CW, in mm) for females with one or more ejaculates in at least one spermatheca and for Group II new and old recruits sampled from April to December 1991. Slopes and elevations were compared by analyses of variance and covariance, respectively. Adjusted equations were fitted to the data after equality of slopes was demonstrated. r^2, coefficient of determination; F, F-statistic; ns, $P > 0.2$; ***$P < 0.001$; N, sample size.

Female	Regression equation	r^2	F	N
1 ejaculate	log FEC = 2.407 log CW + 0.342	0.508	104.37***	103
>1 ejaculate	log FEC = 2.725 log CW – 0.136	0.707	465.72***	195
Slopes			1.39 ns	
Elevations			32.64***	
Adjusted equations				
1 ejaculate	log FEC = 2.656 log CW – 0.094			
>1 ejaculate	log FEC = 2.656 log CW – 0.016			
New recruit	log FEC = 2.948 log CW – 0.633	0.612	115.30***	75
Old recruit	log FEC = 2.506 log CW – 0.255	0.690	176.14***	81
Slopes			1.58 ns	
Elevations			51.78***	
Adjusted equations				
New recruit	log FEC = 2.616 log CW – 0.050			
Old recruit	log FEC = 2.616 log CW + 0.062			

ductive cycle. First, in April 1991 and in March 1992, about 2 mo before the peak hatching time, most females and in particular old recruits carried eggs without eyespots (Fig. 1 and 2). Second, after 11 mo of development from April 1991 to March 1992, new recruits had ovaries that were less than half the weight of ovaries of females with mature broods and eggs that had not yet developed eyespots (Fig. 2). Third, at any given time, up to two or three groups of females could be separated on the basis of apparent age and egg development (Fig. 2). The progression of adjusted weight of ovaries and of brood stage for these groups of females produced a coherent, biennial pattern (Fig. 2 and 3). Finally, spermathecae of Group II females contained twice as much recent ejaculate as those of Group III females, whereas both groups exhibited a significant trend of decreasing ejaculate weight from April 1991 to March 1992 (Fig. 4). This observation independently supports the separation between Group II and Group III females and implies that the latter were inseminated at a much earlier time than the former.

The reproductive cycle inferred for female *C. opilio* in Baie Sainte-Marguerite is the following. The main period for egg extrusion is February–April for primary spawners (this study; Sainte-Marie and Hazel 1992, and unpublished data) and April–June for repeat spawners. Brood and ovary development are phased, as indicated by the positive partial correlation between egg diameter and weight of ovaries (Table 2) and scarcity of barren females (Fig. 1). Since egg hatching occurs from April to June (Fig. 1), maturation of ovaries and brood lasts approximately 27 mo in primiparous and approximately 24 mo in multiparous females (Fig. 2 and 3). Thus, primiparous females in Groups I, II, III, and IV spawned in 1992, 1991, 1990 and 1989, respectively. Eggs remain without eyespots for approximately 16–19 mo; eyespots appear only in the fall of the second year of brooding. This protracted development may result from diapause early in the incubation period, such as documented for two other majid species by Wear (1974). The times for egg extrusion and hatching in Baie Sainte-Marguerite are the same as those inferred or observed for other localities of the Gulf of Saint Lawrence (Brunel 1962; Powles 1968; Watson 1969, 1970, 1972; Elner and Robichaud 1983; Elner and Gass 1984; Taylor et al. 1985; Conan and Comeau 1986; Hooper 1986; Moriyasu et al. 1987; Ennis et al. 1988, 1990; Comeau et al. 1991). However, the 2-yr development period of eggs in Baie Sainte-Marguerite is much longer than the previously estimated 18 mo for primiparous and 12 mo for multiparous females, a pattern often surmised to be general (e.g., Adams 1979; Elner and Beninger 1992).

Field evidence in support of an annual reproductive cycle for *C. opilio* in eastern Canada is not overwhelming. It revolves around Watson's (1969) time series of egg development in females from unspecified localities in the Gulf of Saint Lawrence, presumably Baie des Chaleurs and the Gaspé coast. Watson (1969) reported that all mature females carried eyed or hatching eggs from January to April inclusively, which admittedly implies annual breeding and contrasts sharply with my observations (Fig. 1 and 2); however, the proportion of females with eyed eggs in January and February was guessed, and sample sizes and collection depths and methods were not specified. This is important because females segregate spatially by reproductive experience (Sainte-Marie and Hazel 1992), and catchability in traps may change with brood development. Other authors have simply inferred a 1-yr cycle because there are annual, synchronous periods of egg extrusion and hatching (e.g., Powles 1968; Elner and Robichaud 1983; Coulombe 1984). Brunel (1962) also noted this, but cautiously offered that whether egg development lasts 1 yr or more cannot be settled on the basis of knowledge of extrusion and hatching periods alone. A major influence on Canadian scientific opinion, undoubtedly, was the early Japanese literature which reported an approximately 1-yr reproductive cycle (e.g., Ito 1963, 1967; Kon and Nanba 1968; Kon and Honma 1970; Kon 1980). However, there is now convincing data in support of a 2-yr cycle for *C. opilio* in the southwest Okhotsk Sea (Kanno 1987) as well as for queen crab, *C. japonica* in the Japan Sea (Ito 1976).

This is the first study to have considered a year-long time series of ovary and brood development for *C. opilio* in eastern Canada. However, Elner and Gass (1984) qualitatively investigated the degree of ovary and egg development for a population off northwest Cape Breton in November 1983. They found that some females had large ovaries and eggs with eyespots, while others

had small ovaries and eggs without eyespots. This difference in development was tentatively explained by the presence of primiparous and multiparous females with 18- and 12-mo spawning cycles, respectively. But this seems unlikely, since all females had dark exoskeletons, similar wear patterns, and widespread bryozoan mats. The pattern observed by Elner and Gass (1984) is similar to that seen in Baie Sainte-Marguerite in December 1991 (Fig. 2). In my opinion, more investigations of *C. opilio* in eastern Canada are necessary to ascertain the existence of the 1-yr reproductive cycle, and if it indeed does exist, to measure the scope of annual and biennial reproductive patterns with respect to oceanographic and demographic features.

Brood Characteristics and Reproductive Status

Fecundity and egg size of female *C. opilio* in Baie Sainte-Marguerite were comparable with those reported in previous investigations of Gulf of Saint Lawrence populations (e.g., Brunel 1962; Watson 1969; Haynes et al. 1976; Davidson et al. 1985). Only limited comparisons are possible, however, because earlier work did not discriminate between primiparous and multiparous females.

A high proportion of the largest females, all multiparous, carried broods that were abnormally small (Fig. 5). Similarly, Kon (1974) reported that egg loss was greater in large than in small females of *C. opilio* from the Japan Sea. Reduced fecundity and/or brood mortality may result from a variety of processes (Kuris 1991). However, only failure to extrude and egg loss by abortion are know to selectively affect large females of decapod species (Kuris 1991), and infertility is their most probable cause. Female infertility could result from a reduction in the availability of suitable mates in crustacean populations subjected to high male fishing mortality (McMullen and Yoshihara 1971; Smith and Jamieson 1991). Paul (1984) showed that multiparous females of *C. bairdi* that were forced to use stored sperm to produce second and third clutches were less fecund than similar females that were allowed to mate prior to extrusion. In the northwest Gulf of Saint Lawrence, fishing mortality of large *C. opilio* males is estimated at 60–70% (Dufour and Coutu 1989).

Two novel observations on sperm transfer and storage reported herein require some discussion. First, the weight of recent ejaculate remaining in spermathecae shortly after brood extrusion was twice as great in primiparous as in multiparous females. This may be due to the fact that the spermathecae have more room to expand (Diesel 1989) in the vacuous body cavity of recently moulted, mature females than in the tightly filled body cavity of hard-shelled, repeat spawners. Complementary or alternative explanations are (1) reduced use of sperm by primiparous females due to their relatively smaller broods, (2) limited room in the spermathecae of multiparous females due to previously stored ejaculatory products, and (3) assortative mating with males with different reproductive potentials. Second, my data indicate that the quantity of ejaculate in spermathecae decreases over time, independently of spawning events. This observation may have implications for Paul's (1984) estimates of sperm required to fertilize broods in *C. bairdi*. Those estimates were based on the decrease in volume of stored ejaculate, assuming that number of sperm per unit volume of ejaculate was constant and that changes in the volume of ejaculate resulted only from expenditure of sperm at time of extrusion. The presently observed decrease in weight of recent ejaculate may be due only to reduction of seminal fluids, although the fate of spermatophores and sperm remains unknown.

Excluding females with abnormally small broods, my data indicate no significant brood mortality over time for primiparous and multiparous females of *C. opilio* in Baie Sainte-Marguerite. Indeed, fecundity in both types of females was independent of weight of ovaries or of elapsed time for a standard carapace width. This finding contrasts with an earlier report of ~54% brood mortality in *C. opilio* females from the Japan Sea (Kon 1974). This latter estimate, however, was based on one comparison in time of the fecundity of females in two different year classes, one sampled prior to and the other soon after extrusion, and the proportions of primiparous and multiparous females in samples were not determined. In the western Gulf of Saint Lawrence, Brunel (1962) found that females ~65–80 mm CW carried about 30 000 to 85 000 orange eggs but only 10 000 to 30 000 ripe, eyed eggs (see his fig. 1). However, he stated that hatching had already started in many of the females with eyed eggs; thus, no brood mortality can be inferred from his data.

Brood size and fecundity were less in primiparous than in multiparous females for a constant carapace width (Table 3). Such a difference was suspected for *C. opilio* (Haynes et al. 1976; Jewett 1981; Elner and Robichaud 1983), but never demonstrated. Brood size and fecundity of females are constrained by the volume of body cavity available for development of ovaries (Hines 1982, 1992; Somerton and Meyers 1983), which obviously is less before than after the moult to maturity. Therefore, knowing that brood weight and fecundity scale isometrically to body weight (this study; Hines 1982, 1992) and that the increment in carapace width at terminal moult of female *C. opilio* in Baie Sainte-Marguerite is ~16–18% (Sainte-Marie and Hazel 1992, and unpublished data), one may predict from Eq. 2 that primiparous females should be ~63–66% as fecund as multiparous females. The estimated differences from my field samples, however, were much less: adjusted fecundity of primiparous females was 77.3–83.6% that of multiparous females. By comparison, Somerton and Meyers (1983) reported that primiparous females of *C. bairdi* were ~70% as fecund as equal-sized multiparous females.

For *C. opilio* there may be at least two reasons for the difference between predicted and observed ratios of primiparous to multiparous fecundity. One is that some females were misclassified, a particularly acute problem when female type was defined by number of ejaculates in spermathecae. Misclassified females were those that (1) extruded a second brood without mating or (2) remated before releasing their first brood. Such cases occur in *C. opilio* and are documented for other majids as well (Hinsch 1968; Schöne 1968; Diesel 1989). Furthermore, if males curette spermathecae prior to insemination, as proposed (Beninger et al. 1991; Elner and Beninger 1992), the number of stored ejaculates may not reflect the number of matings. Errors in classification were less likely to occur when apparent age was used to discriminate between primiparous and multiparous females, hence the greater contrasts in reproductive traits. The second reason, a biological one, is that some multiparous females do not realize their full reproductive potential due to fertility problems (see above).

Eggs of primiparous females were slightly, albeit significantly, larger than those of multiparous females. This novel observation is consistent with the approximately 3-mo longer development period of eggs in primiparous compared with multiparous females. Indeed, development period is known to depend on egg

size for a variety of crustaceans (e.g., Wear 1974; Steele 1977). The consequences of this size difference for larval development and survival are unknown. Interestingly, Roff et al. (1984) noted that *C. opilio* zoeae II on the Scotian Shelf had a bimodal size distribution.

Number of Broods and Lifetime Fecundity

Females of *C. opilio* in Baie Sainte-Marguerite probably carry only two broods in a lifetime. Because mature females are terminally moulted (this study; Yoshida 1941; Ito 1963; Watson 1970), the number of broods produced by a female depends on life expectancy following the moult to maturity. Comeau et al. (1991) reported that mature females in Bonne Bay, Newfoundland, live for a maximum of 4 yr after the terminal moult, and the oldest exoskeletons in the Saguenay Fjord (Sainte-Marie et al. 1992) are ~5 yr (G.Y. Conan, Department of Fisheries and Oceans, Gulf Region, Moncton, N.B., personal communication), based in both cases on ageing by radioelements. If this is also the case for Baie Sainte-Marguerite females, then a 4- to 5-yr life span after maturity and a 2-yr reproductive cycle allow hatching of only two broods. The number of ejaculates in spermathecae appears to bear out this conclusion: only a small minority of females had two or more ejaculates per spermatheca. However, counting of ejaculates to determine maximum number of broods may be unreliable, for reasons detailed above.

Biennial breeding and biparity have important implications. Lifetime fecundity for *C. opilio* in Baie Sainte-Marguerite averages 81 630 to 83 143 eggs for a standard 57.4 mm CW female. Assuming conservatively that primiparous females are only 63–66% as fecund as multiparous females, and that there is no brood mortality, primiparous females would account for >40% of larvae from the Baie Sainte-Marguerite population. This estimate, however, assumes that there is no mortality of mature females. My findings also imply that small males contribute extensively to reproduction. Indeed, 88.3% of pubescent females in Baie Sainte-Marguerite mated with morphometrically mature males smaller than the minimum legal size of 95 mm CW (Sainte-Marie and Hazel 1992). Moreover, a substantial proportion of repeat spawners apparently relied on stored sperm from the pubescent mating (this study), and an additional unknown proportion may have remated with small morphometrically mature males (e.g., Ennis et al. 1990). This is disquieting, since morphometric maturity is achieved only at the terminal moult of males (Conan and Comeau 1986; Sainte-Marie and Hazel 1992) and might be genetically determined (Conan 1986; Elner et al. 1986; Bailey and Elner 1989).

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An in situ Evaluation of the Day-to-Day Variation in the Quantity of Food Consumed by Fish

Marc Trudel[1] and Daniel Boisclair

Université de Montréal, Département de sciences biologiques, C.P. 6128, Succursale « A », Montréal, QC H3C 3J7, Canada

and

Station de Biologie des Laurentides de l'Université de Montréal, 440, avenue du Lac Croche, C.P. 159, St-Hyppolyte, QC G0R 1P0, Canada

Trudel, M., and D. Boisclair. 1993. An in situ evaluation of the day-to-day variation in the quantity of food consumed by fish. Can. J. Fish. Aquat. Sci. 50: 2157–2165.

We tested the hypothesis that the mean quantity food consumed by fish in situ does not vary significantly over successive days. The daily ration of minnows (*Phoxinus eos* × *P. neogaeus*) was estimated over 5–12 consecutive days (June 1–5, July 6–17, and August 3–9, 1992). Mean daily ration ranged from 0.60 to 1.32 g dry·100 g wet^{-1}·d^{-1} and varied significantly over consecutive days in June and July, but not in August. Average day-to-day variation in food consumption rates ranged from 7.0 to 16.3%. Mean daily ration was not influenced by either water temperature or percent cloudiness. Simulated long-term consumption rates of minnows did not vary by more than 19.3% among sampling intervals ranging from 1 to 30 d. We conclude that long-term consumption rates can be accurately determined using time series of daily ration estimated at 3- to 4-wk intervals.

Nous avons testé l'hypothèse selon laquelle la quantité de nourriture consommée in situ par les poissons ne varie pas significativement au cours de journées consécutives. La consommation journalière de ménés (*Phoxinus eos* × *P. neogaeus*) a été estimée durant 5 à 12 jours consécutifs (1–5 juin, 6–17 juillet, et 3–9 août 1992). La consommation journalière moyenne a varié entre 0,60 to 1,32 g sec·100 g humide^{-1}·jour^{-1}. Ces variations ont été statistiquement significatives entre les journées de juin et juillet, mais pas entre celles d'août. La variation journalière moyenne s'est situé entre 7,0 et 16,3 %. La consommation journalière n'a pas été influencée par la température de l'eau ou l'ensolleillement. La consommation à long terme des ménés n'a varié que de 19,3 % entre des intervalles d'échantillonnage s'échelonnant de 1 à 30 jours. Nous concluons que la consommation saisonnière peut être adéquatement déterminée en utilisant une série temporelle de rations journalières estimées à toutes les 3–4 sem.

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Food consumption has been used to explain variations in fish growth (Boisclair and Leggett 1989a) and mortality rates (Adams et al. 1982; McGurk 1984), to support the existence of competition among fish species and size classes (Persson 1983, 1986; Hanson and Leggett 1986), and to predict the impact of fish on their prey community (Stewart et al. 1983; Ruggerone 1989; Madenjian and Carpenter 1991). Consumption rates are often expressed as integrated measures of the quantity of food consumed over extended periods of time (e.g., month, season, year). Such long-term comsumption rates can be determined using either the punctual or the bioenergetic approach.

The punctual approach is based on the determination of a series of daily ration values, each requiring the estimation of the quantity of food found in the stomach or complete digestive tract of fish over 1d and the estimation of the evacuation rate during that day (Eggers 1977; Elliott and Persson 1978; Boisclair and Leggett 1988; Parrish and Margraf 1990; Boisclair and Marchand 1993). An integrated measure of food consumption is obtained by calculating the area under the curve defined by the time series of daily ration values (Persson 1983; Hayward and Margraf 1987; Boisclair and Leggett 1985, 1989a). Daily ration values are usually estimated at weekly to monthly intervals (Boisclair and Leggett 1985, 1989a; Hayward and Margraf 1987). Consequently, the accuracy of long-term food consumption rates determined using the punctual approach depends on the assumption that fish daily ration does not vary substantially over consecutive days.

The bioenergetic approach calculates consumption rates as the difference between growth and a suite of metabolic costs and losses (Kitchell et al. 1977). This method is based on the estimation of fish weight and growth, water temperature, prey and fish caloric density, and the value of the undigestible fraction of the prey in the diet (Hewett and Johnson 1987, 1992). Furthermore, the accuracy of food consumption estimates derived from bioenergetic models depends on the accuracy of 20–30 model paramaters and on untested assumptions regarding fish activity costs (Stewart et al. 1983; Stewart and Binkowski 1986; Carline 1987; Beauchamp et al. 1989; Hewett and Johnson 1992).

The validity of both sets of assumptions, and hence the accuracy of estimates derived using both approaches, has been criticized. Smagula and Adelman (1982) showed that food consumption rates estimated under laboratory conditions may

[1]Present address (as of January 1994): Department of Biology, McGill University, 1205 ave. Dr. Penfield, Montreal, QC H3A 1B1, Canada.

vary substantially from day to day. Boisclair and Leggett (1989b) used the comparative field approach and suggested that activity cost can represent a large and variable proportion of the fish energy budget (0–40%). Although there is some circumstantial evidence supporting the hypothesis that the average quantity of food consumed by fish in the field does not vary substantially over consecutive days (Nakashima and Leggett 1978; Diana 1979), studies performed to specifically test this hypothesis have not been conducted under natural conditions (Rozin and Mayer 1961; Smagula and Adelman 1982).

The purpose of this paper is to present the results of the first field study designed to directly test the hypothesis that the mean quantity of food consumed by fish does not vary significantly over consecutive days. The influence of day-to-day variation and sampling frequency on long-term consumption rates is also evaluated.

Methodology

Estimation of the quantity of food consumed by fish during 1d requires data on the diel variation in their digestive tract contents and the determination of their evacuation rate during that day. In this study, diel variations of fish digestive tract contents were estimated in the field, while evacuation rate was estimated under laboratory conditions.

Species and Sampling Site

The study was performed using hybrids of northern redbelly dace and finescale dace (*Phoxinus eos* × *P. neogaeus*). Sampling was done in Lake Triton (45° 59′N, 74°00′W), a small (area = 1.8 ha; maximum depth = 3.6 m) oligotrophic lake located at La Station de Biologie des Laurentides de l'Université de Montréal (70 km northwest of Montréal), in the lower Laurentian region of Québec. This minnow and the pumpkinseed (*Lepomis gibbosus*) are the only fish species present in Lake Triton. This lake was selected because preliminary sampling suggested that its high minnow biomass and numerical density could sustain the intense sampling regime required by our work.

Field Procedure

Sampling

Consumption rates were estimated on 5–12 consecutive days during the months of June (June 1–5), July (July 6–17), and August (August 3–9) 1992. Each of these time intervals is further referred to as series I, II, and III, respectively. Fish were collected at 6-h intervals beginning at 07:00 using one to three minnow traps (80 × 32 cm; 0.36-cm^2 mesh) during daytime and using a knotless nylon seine (20 × 2 m; 0.2-cm mesh) at night. Minnow traps were never set for more that 15 min. The locations of the traps and the seining sites were randomized within the lake. Sampling effort was kept constant among sampling days. Sampling performed on May 29 and July 2, 1992, indicated that the mean quantity of food found in the complete digestive tract of minnows captured in traps was not significantly different from that of minnows sampled simultaneously by seining ($t_{18} = 0.339$; $p > 0.7$; mean difference = 9.0% and $t_{18} = -0.143$; $p > 0.8$; mean difference = 2.6%).

Minnows were killed by an overdose of 2-phenoxyethanol (1 ml·L^{-1}) immediately following capture. Ten fish ranging from 40 to 70 mm total length were randomly selected from each sample and retained to estimate daily ration. Aging performed using scales (Frost 1943) indicated that 91% ($n = 45$) of minnows of this size were age 3+ fish. Total blotted weight was recorded (±0.01 g). Total digestive tract contents (no well-defined stomach is present in this species) were removed within 1h of capture, dried (60°C for 24 h), and weighed using an electronic balance (±0.0001 g).

Fish diet

Ten additional fish were collected on June 29, July 9 and 26, and August 2 and 26, 1992, to describe fish diet. The complete digestive tract contents of each fish were removed and placed in a Ward cell to facilitate counting and identification of prey. The relative contribution of each prey type to the diet of a fish was expressed as a percentage of the total number of prey consumed by that fish.

Environmental conditions

Water temperature (degrees Celsius) from the surface to a depth of 3 m at the deepest part of the lake and weather condition were recorded every day. Weather conditions were divided into three categories: sunny (percent cloudiness <20%), partly sunny (percent cloudiness from 20 to 80%), and rainy (percent cloudiness 80% plus rain).

Laboratory Procedure

Evacuation rate of minnows was determined in the laboratory. Fifty minnows (40–60 mm) were introduced into an aquarium (40 L) equipped with a filter. Water temperature was controlled by placing the aquarium in an incubator. The fish were kept at 19.5°C for at least 2 wk before the experiment. This temperature was selected because it represented the average water temperature observed in July and August 1992 in the field. Brine shrimp (Anostraca: *Artemia*) were provided once daily to mimic the feeding schedule and diet in the field (see Results).

Complete digestive tract contents were examined beginning 1 h after feeding. Five fish were killed at 4-h intervals for 20 h. Fish weight and the total weight of digestive tract contents were noted following the prodedure used in the field.

Computations and Data Analysis

Digestive tract contents

The weight of the digestive tract contents relative to the weight of the fish (F_i, grams dry per 100 g wet; further referred to as gut fullness) was calculated for each dissected fish as

$$(1)\quad F_i = \frac{G_i}{W_i} \cdot 100$$

where G_i (grams dry) is the weight of the digestive tract contents of fish i and W_i (grams wet) is the weight of that fish. Prior to analysis, individual gut fullness values were arcsin $(\sqrt{F_i/100})$ transformed. This transformation was successful in eliminating heteroscedasticity and is recommended to normalize percentages (Sokal and Rohlf 1981; Boisclair and Leggett 1988). Mean gut fullness values among sampling days within a given series were compared using two-way analysis of variance (ANOVA).

Evacuation rate

The following exponential model was used to estimate the evacuation rate R (Elliott 1972):

$$(2)\quad F_T = F_0 \cdot e^{-RT}$$

where F_0 is the initial mean gut fullness and F_T is the mean gut fullness at time T. The gut fullness of each fish was transformed using the natural logarithm, so that R and its standard error could be estimated by simple linear regression.

Consumption rate

Mean daily ration (C_d, grams dry per 100 g wet per day) was calculated using the Eggers (1977) model as modified by Boisclair and Leggett (1988):

$$(3) \quad C_d = F_d \cdot R \cdot 24$$

where F_d is the mean gut fullness (back-transformed from arcsin $(\sqrt{F_i/100})$, grams dry per 100 g wet) on day d, R is the evacuation rate (per hour), and 24 is the number of hours in a day.

Estimation of the error associated with daily ration values requires simulations that account for the error around mean gut fullness F_d and evacuation rate R. This objective can be achieved using two approaches: (1) combining a "pseudo-value" of mean gut fullness assuming a particular distribution with a "pseudo-value" of evacuation rate (assumed to be normally distributed because R is determined as the slope derived by linear regression) and (2) combining actual individual gut fullness values with a "pseudo-value" of evacuation rate. These two approaches will give similar results because the distribution of gut fullness (to be used following the first approach) is determined using individual gut fullness indices (to be used in the second approach). In our work, we used the second approach because it obviates the need to assume that the distribution that would have been used following the first approach accurately represents the original data.

To account simultaneously for the error associated with "F_d" and "R," we used a simulation technique derived from the bootstrap method (Efron and Tibshirani 1986) to estimate daily consumption rate (C_i, grams dry per 100 g wet per day) as follows:

$$(4) \quad C_i = F_i \cdot R^* \cdot 24$$

where F_i is the gut fullness of fish i and R^* is a pseudo-value of evacuation rate. This operation was performed for every fish. R^* was calculated as

$$(5) \quad R^* = R + (SE_R \cdot RN)$$

where R is the evacuation rate, SE_R is the standard error of R, and RN is a normally distributed random number having a mean of 0 and a standard deviation of 1. A different RN was generated each time an R^* value was calculated.

We tested the significance of the difference among consumption rates obtained during a given series using one-way ANOVA. Individual consumption estimates were arcsin $(\sqrt{C_i/100})$ transformed prior to analysis. This approach is similar to the use of the median (Hayward 1991), but allows the calculation of the variance of normally distributed consumption rates. This operation therefore facilitates the statistical comparison of different consumption rate estimates by means of parametric techniques. When significant differences were found among consumption rates within a series, multiple comparisons tests (Turkey's HSD, SNK) were performed (Sokal and Rohlf 1981).

For each series, day-to-day variation (DTDV, percent) in the quantity of food consumed by fish was calculated as

$$(6) \quad \text{DTDV} = \frac{|C_d - C_m|}{C_m} \cdot 100$$

where C_d is the mean daily ration on day d and C_m is the average consumption rate of fish during that series.

Influence of Sampling Interval on Cumulative Consumption Rates and Associated Precision

We tested the influence of DTDV and sampling frequency on long-term consumption rates by using resampling techniques to construct a 60-d time series of daily ration and estimating cumulative consumption during this period. A time series of 60 daily rations was constructed from the 24 consumption rates estimated during this study. The 60-d period was assumed to begin on June 1 and end on July 30. One of the 24 mean daily ration values with its standard error was assigned randomly to each day where no consumption rate had been estimated. This approach assumes independence between fish daily consumption rates. Statistical analyses based on Geary's c and Moran's I (Legendre and Legendre 1984) supported our assumption of independence among daily ration values within the July time series. Our sample size was insufficient to test the presence of autocorrelation during the June and August series.

Cumulative consumption by minnows during the 60 d and its 95% CI, was estimated using Monte Carlo simulations. The influence of sampling interval (SI) on cumulative consumption was assessed by calculating the area under the curve defined by pseudo-values of daily ration obtained at different SI (1, 2, 3, 4, 5, 6, 10, 12, 15, 20, and 30 d). For each SI, 1000 series of pseudo-values of daily ration were generated. Mean daily rations were generated independently for each sampling day as follows:

$$(7) \quad C_d^* = C_d + (SE_C \cdot RN)$$

where C_d^* is a pseudo-value of mean consumption rate on day d, C_d is the mean daily ration, SE_C is the standard error of the mean daily ration, and RN is a normally distributed random number having a mean of 0 and a standard deviation of 1. The mean and the 95% CI of cumulative consumption rate were defined, respectively, by the 50 percentile and by the 2.5 and 97.5 percentiles of the 1000 time series of pseudo-values of daily ration. Cumulative consumption rates obtained using two SI were declared significantly different when their 95% CI did not overlap. Seasonal consumption rate obtained at a SI of 1 d served as a reference for comparison of all SI.

Results

Fish Weight

Mean fish weight (back-transformed from the natural logarithm) ranged from 1.28 (July) to 1.51 g wet (June) and varied significantly among series ($F_{2,957} = 24.92$; $p < 0.0001$). Mean fish weight did not differ significantly among sampling days in series I ($F_{4,195} = 1.75$; $p > 0.1$) and III ($F_{6,273} = 1.68$; $p > 0.1$) but differed significantly among sampling days in series II ($F_{11,468} = 2.06$; $p < 0.03$). Mean fish weight tended to increase by 0.02 g wet·d^{-1} from July 6 to July 17 ($F_{1,10} = 8.02$; $p < 0.02$; $r^2 = 0.45$).

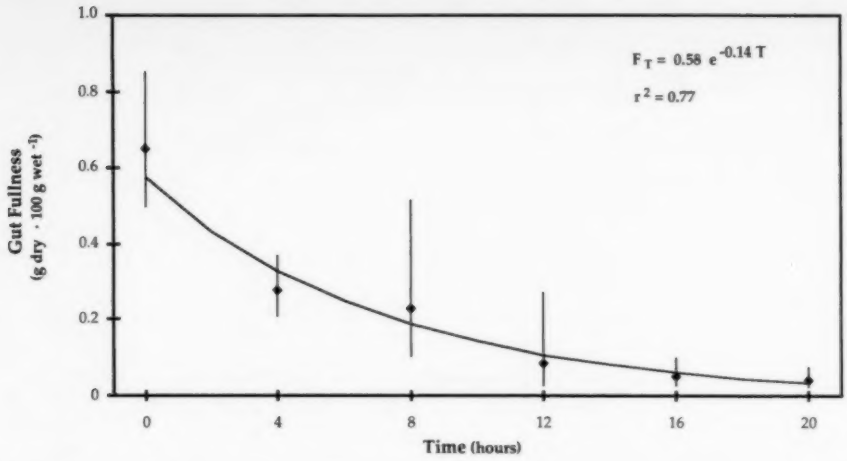

FIG. 1. Observed and fitted decline in the complete digestive tract contents of minnows over time at 19.5°C.

TABLE 1. Statistics of the among-date and among-time-of-day variability in gut fullness (two-way ANOVA).

	F	df	*p*
June 1992			
date	3.28	4,180	<0.02
time	1.78	3,180	>0.1
interaction	1.83	12,180	<0.05
July 1992			
date	5.46	11,432	<0.0001
time	46.40	3,432	<0.0001
interaction	2.51	33,432	<0.0001
August 1992			
date	1.08	6,252	>0.3
time	13.49	3,252	<0.0001
interaction	3.65	18,252	<0.0001

Digestive Tract Contents

Mean gut fullness ranged from 0.17 (July 17) to 0.39 g dry·100g wet^{-1} (July 6). On average, the 95% CI for mean daily gut fullness indices was ±22.0% of the mean values. Mean gut fullness varied significantly among sampling days in series I, II, and III (two-way ANOVA) (Table 1).

Evacuation Rate

The evacuation rate R of the whole gut at 19.5°C was 0.14·h^{-1} ($F_{1,28} = 92.93$; $p < 0.0001$; $r^2 = 0.77$) (Fig. 1). The standard error associated with this evacuation rate was 0.01·h^{-1}. The evacuation rate was assumed to be constant during the study.

Food Consumption

Mean daily ration ranged from 0.74 to 1.10 g dry·100g wet^{-1}·d^{-1} for series I and III. Corresponding values for series II were 0.60–1.32 g dry·100 g wet^{-1}·d^{-1} (Fig. 2). The 95% CI around daily ration estimates averaged ±22.3% of the mean values.

TABLE 2. Average contribution of prey types to the diet of minnows from Lake Triton during the summer of 1992.

Date	Zooplankton (%)	Aquatic insects (%)
June 29	50	50
July 9	14	86
July 26	16	84
August 2	38	62
August 26	83	17

Mean daily ration did not vary significantly among sampling days in series III ($F_{6,273} = 0.83$; $p > 0.5$), but differed significantly among sampling days in series I ($F_{4,195} = 3.19$; $p < 0.02$) and II ($F_{11,468} = 3.86$; $p < 0.0001$). Multiple comparisons tests (HSD, SNK) indicated that the difference observed in series I was caused by a significant difference in daily ration on June 2 and 4. Consumption rates on July 6 and 17 were responsible for the significant difference observed among sampling days in series II. Therefore, 0 (series III) to 20% (series I) of all pairwise comparisons of daily ration values were significantly different over consecutive days. Mean daily ration did not vary significantly among series ($H_2 = 1.59$; $p > 0.4$).

DTDV in mean daily ration estimated during series I, II, and III ranged from 1.1 to 23.2, 0.1 to 39.0, and 0.5 to 16.8%, respectively. DTDV averaged 15.9, 16.3, and 7.0% in series I, II, and III, respectively. There was no significant relationship between daily ration ($F_{1,22} = 1.02$; $p > 0.3$) or DTDV ($F_{1,22} = 0.001$; $p > 0.9$) and mean fish weight.

Diet

The diet of minnows was dominated by aquatic insects (Odonata and Ephemeroptera) in July and at the beginning of August, while zooplankton (mainly cladocerans) were the major prey at the end of August (Table 2). Zooplankton and aquatic insects were equally important in the diet in June (Table 2).

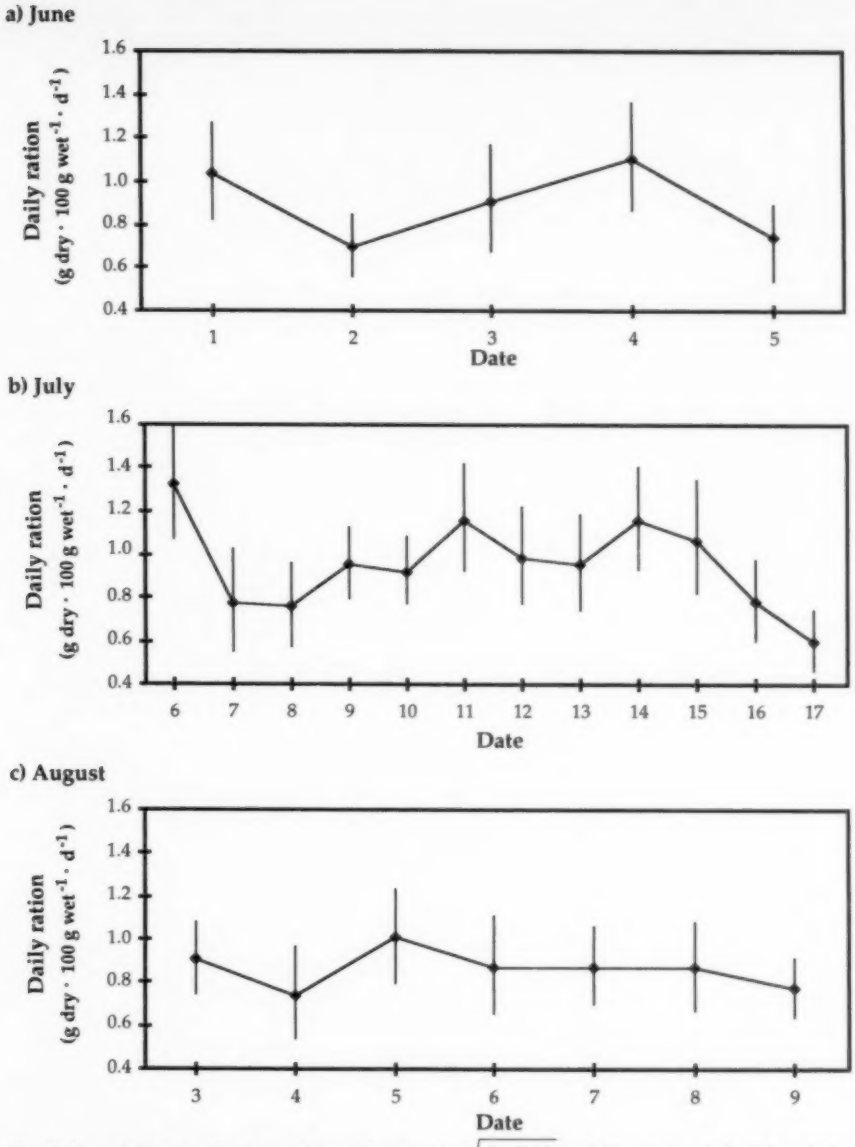

FIG. 2. Mean daily ration (back-transformed from arcsin $(\sqrt{C_i/100})$ of minnows from Lake Triton during consecutive days in (a) June, (b) July, and (c) August 1992. The vertical lines represent the 95% CI of the mean values.

Environmental Conditions

Water temperature ranged from 16.7 (June 1) to 20.7°C (July 11) and differed significantly among sampling days ($F_{23,72} = 1.71$; $p < 0.05$). Average water temperature was 17.6, 19.8, and 19.5°C in series I, II, and III, respectively, and was significantly lower in June ($F_{2,93} = 8.48$; $p < 0.0005$). Mean daily ration was not correlated with water temperature ($F_{1,22} = 0.10$; $p > 0.7$). There was also no significant difference in mean daily ration among weather conditions ($H_2 = 0.69$; $p > 0.7$). Multiple regression analysis also indicated that water temperature and cloud cover failed as predictors of mean daily ration ($F_{3,20} = 0.42$; $p > 0.7$).

Influence of Sampling Interval on Cumulative Consumption Rates and Associated Precision

Cumulative consumption estimated over the period of 60 d using a 1–d sampling interval averaged 837 mg dry (Fig. 3). Long-term consumption estimated using a sampling interval greater than 1 d ranged from 728 (15 d) to 883 mg dry (20 d) (Fig. 3). Cumulative consumption rates obtained at different SI were not

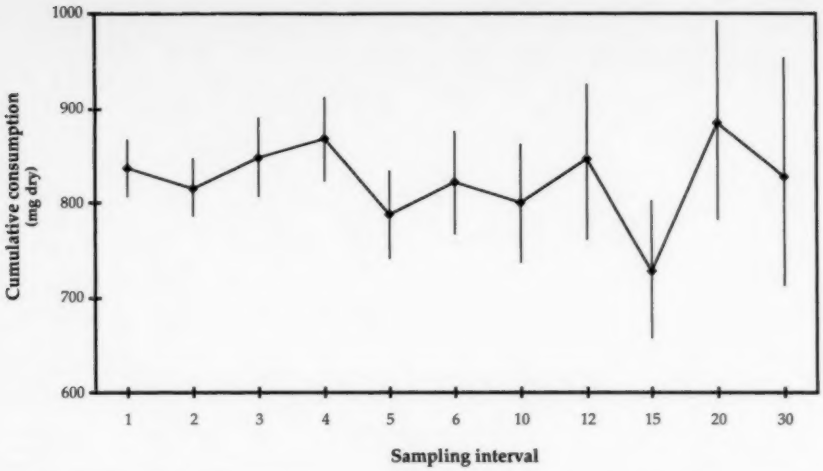

FIG. 3. Cumulative food ingested by minnows during 60 d estimated at different sampling intervals. The vertical lines represent the 95% CI of the mean values.

significantly different from the reference value obtained using the 1-d interval (Fig. 3). One exception to this rule was cumulative consumption rate obtained at a SI of 15 d. The 95% CI ranged from ±3.5 (1 d) to ±14.5% (30 d) of the mean value and tended to increase with the length of the sampling interval (Fig. 3).

Discussion

Our work indicates that the quantity of food consumed by fish does vary significantly over consecutive days. However, our analyses also demonstrate that such variation occurs for a small fraction (0–20%) of all possible paired comparisons of consecutive daily ration values. Furthermore, DTDV generally represented a small percentage of mean daily ration estimated for the three times series examined (10–15%). The low occurrence of significantly different daily consumption rates and the small variation among daily ration values resulted in a relatively small difference (maximum 20%) between long-term consumption rates (cumulative consumption over 60 d) estimated at 1- to 30-d intervals. These results suggest that the punctual approach, which is based on the use of time series of daily ration values to estimate long-term consumption rates, can yield accurate values when daily ration is estimated at 3- to 4-wk intervals. While the only two environmental conditions (water temperature and percent cloudiness) we assessed had no significant influence on DTDV of daily ration, other factors such as current strength and direction, fish size, prey availability, biomass, and taxonomic and size composition could have a significant influence on both daily ration values and variation (Mills and Forney 1981; Mittelbach 1981; Werner et al. 1983; Hayward and Margraph 1987). It is important to note that different levels of environmental variation could result in DTDV either lower or higher than those observed in our study. Clearly, the definition of a more general pattern regarding the magnitude and the influence of DTDV in daily ration on long-term consumption rates requires additional research.

Use of Constant Evacuation Rate

Daily ration estimates presented in this paper are based on whole-gut evacuation rates of minnows fed brine shrimp at 19.5°C. Our evacuation rate is comparable with those obtained by Boisclair and Leggett (1985) for minnow species. Fish evacuation rates are generally believed to be most influenced by water temperature (Sharple et al. 1969; Brett and Higgs 1970; Elliott 1972; Steigenberger and Larkin 1974; Persson 1979, 1981) and prey type (Thorpe 1974, 1977; Griffiths 1976). We believe that our estimate of evacuation rate adequately represents that of fish in the field during July and August because (1) there was a negligible difference between the water temperature used in the laboratory (19.5°C) and that experienced by fish in the field (18.5–20.7°C) and (2) evidence from studies on yellow perch (*Perca flavescens*) suggest that soft-shelled invertebrates (e.g., zooplankton, amphipods) and brine shrimp are evacuated at the same rate (Boisclair and Leggett 1988, 1990). Consumption rate estimates obtained in June, on the other hand, should be lower because water temperature was, on average, 2° lower than that during the digestion experiment. However, because the range of water temperatures was small in June (16.7–18.1°C), evacuation rate should also be nearly constant during these sampling days. Consequently, comparison of consumption rates on a relative basis should yield similar DTDV.

Field-Derived DTDV in Food Consumption Rates

Our results are in agreement with most field-derived evidence on DTDV in the quantity of food consumed by fish. For consistency with our study, published DTDV were recalculated using our equation (6). Nakashima and Leggett (1978) estimated the daily ration of three size classes of yellow perch for 2 consecutive days in July (7–8) and August (6–7) 1975. Their data indicated 10–46% DTDV in the quantity of food consumed (their table 1.) However, their method of estimating fish daily ration is questionable (Cochran 1979). If we assume that variation in gut fullness indices is an accurate indicator of variation in daily ration (i.e., assume a constant evacuation rate), more realistic measures of DTDV in food consumption derived from

TABLE 3. Mean DTDV (%) for different combinations of time series length (d), fish size, and water temperature (°C). Small fish = 108–113 mm; large fish = 136–140 mm. Original data from Smagula and Adelman (1982).

	Series length			
	5	10	15	20
Small fish (14°C)				
Mean	35.0	35.5	35.8	35.9
Minimum	18.7	21.2	24.8	28.3
Maximum	56.1	45.8	45.6	46.1
Small fish (20°C)				
Mean	36.7	39.9	42.2	43.0
Minimum	12.1	21.6	26.6	32.0
Maximum	79.7	65.6	54.2	48.2
Small fish (26°C)				
Mean	20.3	23.4	25.2	25.6
Minimum	6.9	10.5	16.2	22.4
Maximum	34.6	35.1	31.4	27.7
Large fish (14°C)				
Mean	25.7	29.4	32.9	34.5
Minimum	8.6	17.4	24.7	29.8
Maximum	41.7	40.1	38.3	40.5
Large fish (20°C)				
Mean	35.8	37.9	39.7	39.9
Mininum	16.9	22.0	31.7	33.8
Maximum	62.6	50.4	48.1	50.6
Large fish (26°C)				
Mean	21.1	21.8	21.7	22.0
Minimum	12.8	16.1	17.8	19.6
Maximum	33.7	27.0	25.0	24.7

Nakashima and Leggett (1978) could range from 5 to 30% (their Fig. 3). Diana (1979) estimated food consumption rates of the northern pike (*Esox lucius*) for 3 consecutive days and found an average DTDV of 6%. Tackett et al. (1988) estimated the daily food consumption of channel catfish (*Ictalurus punctatus*) over 148 d. Fish were stocked in 0.25-acre ponds with a "demand-feeder" containing "floating catfish feed." They noted 50–70% variation in average daily ration. Differences between their results and those of previous workers might be related to the estimation of fish daily ration using only the quantity of food delivered by the feeder, apparently neglecting the ingestion of natural food items (e.g., benthic invertebrates). Furthermore, it can be hypothesized that hierarchy among fish, stochastic events (e.g., fish distribution), and the capacity of fish to locate the feeder may cause some variation in the availability of the feeder to the individuals in the population, and consequently, of estimated food consumption rates.

Laboratory-Derived DTDV in Food Consumption Rates

Laboratory-derived variations in the quantity of food consumed by fish are generally larger than those determined under field conditions. For instance, Rozin and Mayer (1961) noted that food consumption rates of goldfish (*Carassius auratus*) isolated in tanks varied by approximately 45% over 10–16 consecutive days. Cui and Wootton (1988) used a similar approach with minnows (*Phoxinus phoxinus*) and found DTDV in food consumption rates in the order of 25% (21 d). Smagula and Adelman (1982) fed largemouth bass (*Micropterus salmoides*) with fathead minnows (*Pimephales promelas*) and found that food consumption rates varied, on average, by approximately 30–40% over 36 consecutive days. De Silva and Gunasekera (1989) fed tilapia (*Oreochromis niloticus*) with pellets twice a day until they judged that satiation was attained. Daily ration varied by approximately 40% over 51–70 consecutive days.

Comparison between Field and Laboratory Studies

Published DTDV in the quantity of food consumed by fish under laboratory conditions generally ranges from 30 to 40%. However, both circumstantial evidence based on in situ observations (Nakashima and Leggett 1978: Diana 1979) and our study indicate that average values ranging from 10 to 15% may be more appropriate for field situations. Two hypotheses could explain the discrepancy between laboratory- and field-derived estimates of DTDV. First, longer time series of daily ration values (typically observed in laboratory studies) could yield higher DTDV. This hypothesis was tested by estimating DTDV from time series of different lengths (5, 10, 15, and 20 d) using the data of Smagula and Adelman (1982). These authors estimated daily ration of two size classes of largemouth bass at three temperatures for 36 consecutive days in the laboratory (a total of six experiments). This represents the longest laboratory-derived time series for which data could be obtained directly from a published paper. We estimated DTDV independently for each experiment. Within each experiment, we estimated DTDV using every possible combination of consecutive days for each

time series length. The range of average DTDV tended to be higher in the short time series (Table 3). However, the grand mean DTDV tended to be similar for time series of different lengths (Table 3). This analysis suggests that the length of a time series may not explain a major fraction of the two- to threefold difference between laboratory and field estimates of DTDV. Second, intrinsic characteristics of laboratory studies may lead to higher DTDV. Under laboratory conditions, the sample size is generally smaller (often 1 and always less than 15) than that used in the field (40 for our study). Laboratory studies may therefore be more suitable for determining individual rather than population variations. In addition, fish are often fed to satiation, either under all-or-none feeding schedules or with large prey. Because the digestion of a relatively large meal or prey may require more that 24 h, daily ration determined under these conditions can a priori be expected to vary substantially among consecutive days (Boisclair and Leggett 1989b). These comparisons suggest that the influence of DTDV in consumption rates on in situ long-term consumption values should be performed using field-derived measures of DTDV in food intake.

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An Appraisal of the Size-Dependent Mortality Hypothesis for Larval Fish: Comparison of a Multispecies Study with an Empirical Review[1]

Pierre Pepin

Department of Fisheries and Oceans, Science Branch, P.O. Box 5667, St. John's, NF A1C 5X1, Canada

Pepin, P. 1993. An appraisal of the size-dependent mortality hypothesis for larval fish: comparison of a multispecies study with an empirical review. Can. J. Fish. Aquat. Sci. 50: 2166–2174.

This study presents an assessment of the size-dependent mortality hypothesis for larval fish from a multispecies survey of Conception Bay, Newfoundland, Canada. Mortality rates are estimated using a length-based method (per millimetre). The results from this survey are consistent with previous studies which indicate that losses decrease with increasing size of fish. However, for each species within this survey, mortality rates are constant. Comparison of mortality rates within species among surveys indicates that as the range of size categories sampled increases, the estimated mortality rates decrease, despite evidence of adequate fit to the length-based model. The findings indicate that previous relationships between size or stage and mortality of larval fish should be reevaluated. Length-based methodology used to estimate mortality rates of larval fish appears to provide biased estimates of this vital characteristic. It is suggested that using size as a proxy for biological age (i.e., assuming a constant growth rate) may be an invalid assumption. Future surveys will need to provide accurate information about the age structure of larvae sampled in order to properly estimate mortality rates.

Nous avons évalué l'hypothèse selon laquelle la mortalité serait dépendante de la taille chez les larves de poissons à partir d'un relevé plurispécifique mené dans la baie Conception, à Terre-Neuve (Canada). Nous estimons les taux de mortalité par une méthode fondée sur la longueur (par millimètre). Les résultats de ce relevé concordent avec des études précédentes qui indiquaient que les pertes diminuent à mesure qu'augmente la taille des poissons. Toutefois, pour chaque espèce de notre étude, les taux de mortalité sont constants. La comparaison des taux de mortalité au sein d'une même espèce entre les divers relevés indique que, quand l'étendue des catégories de tailles échantillonnées augmente, les taux de mortalité estimés diminuent, malgré la qualité de l'ajustement au modèle fondé sur la longueur. Les résultats indiquent qu'il serait bon de réévaluer les relations précédemment établies entre la taille ou le stade et la mortalité des larves de poissons. La méthodologie fondée sur la longueur utilisée pour estimer les taux de mortalité des larves de poissons semble fournir des estimations biaisées de cette caractéristique essentielle. Il apparaît que le fait d'utiliser la taille en place de l'âge biologique (c.-à-d. en posant que le taux de croissance est constant) peut constituer une hypothèse invalide. Les relevés futurs devront fournir une information exacte sur la structure d'âge des larves échantillonnées pour donner des estimations correctes des taux de mortalité.

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To investigate the causes of variations in survival of fish larvae, it is essential to obtain accurate and precise measurements of both growth and mortality rates (Houde 1987; Beyer 1989; Pepin 1991). Growth rates generally have been measured using cohort analysis based on length frequency distributions (e.g., MacDonald and Pitcher 1979) or using empirical relationships between length and age estimated from counts of daily increments on otoliths (see Campana and Neilson 1985). Mortality rates have been difficult to estimate because of problems in sampling an entire population throughout its early life history. A traditional method of determining larval fish mortality rates involves estimating the slope of the descending limb of the distribution curve describing the relationship between abundance, cumulated over the sampling period, and the length of the larvae (Ahlstrom 1954; Smith and Richardson 1977), herein referred to as a length-based method. This method assumes that the mortality rate is constant over the sampling period (Ahlstrom 1954).

Because of difficulties in obtaining several estimates of larval fish mortality rates from a single species, stock, or location, many attempts have been made to derive general empirical models using a comparison among species to provide a perspective on the major factors that influence this vital characteristic (Peterson and Wroblewski 1984; McGurk 1986; Houde 1989; Pepin 1991). All these reviews showed evidence of size dependency. One may interpret this general pattern as indicating that species with larger larvae, or sizes at hatch, suffer lower mortality rates than species with smaller larvae but that these rates of loss are constant throughout this stage for each species. However, a more general interpretation is that mortality rates of fish larvae decrease with increasing size. The latter is consistent with the general concept that mortality in pelagic marine ecosystems is strongly dependent on the size of organisms (Pauly 1979; Silvert and Platt 1980; Peters 1983, p. 131–133; Peterson and Wroblewski 1984). As larvae grow, their vulnerability to starvation and predation decreases.

[1]This study is a contribution to the Northern Cod Science Program.

Conclusions derived from general empirical models suffer from several limitations. Chief among them is the coalescence of data from a suite of surveys, which can differ in methodological approaches and environmental conditions. Empirical relationships are established among variables that are common to all sources or that are inferred by combining studies of the same system. However, the principle explanatory variable may differ among reviews simply because of uncontrolled factors among the information sources or as a result of alternative perspectives on the part of the investigators. For example, in the case of size-dependent mortality of larval fish, Taggart and Frank (1990) suggested that the time interval between abundance estimates can lead to biases in estimated larval fish mortality rates. As the survey period is lengthened and a greater relative proportion of the early life history is sampled, the average mortality rates appear to decrease. Taggart and Frank (1990) proposed that differential immigration and production in the survey area can result in the observed pattern of mortality rates in relation to sampling period. However, as the survey period is lengthened, the average size of animals sampled also increases. The confounding effects of survey period and animal size are impossible to dissociate unless all populations or species are surveyed using the same methodology and at the same time. Finally, the empirical conclusion that larval fish mortality rates are size dependent is inconsistent with the assumption of a constant rate of loss implicit in the traditional length-based method used to estimate the mortality rates, which form the data base for the general reviews referenced above. One consequence of this inconsistency is that length-based estimates of larval fish mortality rates may be inaccurate and potentially biased. Because of the potential use of general empirical models of size-dependent processes in theoretical developments and in hypothesis building (Miller et al. 1988; Beyer 1989; Houde 1989; Pepin 1991, 1993; Pepin and Miller 1993), there is a need to ascertain whether mortality rates of larval fish are dependent or independent of their size.

In this study, I evaluate the size-dependent mortality hypothesis using the results from a multi-species ichthyoplankton survey of Conception Bay, Newfoundland, Canada, conducted over a 3-wk period. Mortality rates were estimated using the traditional length-based method (Ahlstrom 1954). The fit of this model was studied to assess how well it represents the losses for each species sampled. Results from this survey were contrasted with observations from previous studies, comparisons being made both among and within species.

Materials and Methods

Study Site

Conception Bay (47°45′N, 53°00′W), Newfoundland, Canada (Fig. 1), is approximately 40 km long and 25 km wide with a maximum depth exceeding 300 m. A sill at the mouth of the bay reaches a depth of approximately 150 m. Surface circulation in the bay is driven principally by local winds and by the inner arm of the Labrador current which flows north to south immediately beyond the sill. Currents are variable but generally flow into the bay on the western side and exit the bay on the eastern side (J. Helbig, P. Pepin, E. Colbourne, and R. Laprise, unpublished data). The surface exchange is largely restricted to the upper 50 m of the water column during the summer. There is some exchange of water in the bottom layer but this is of little importance to the present study.

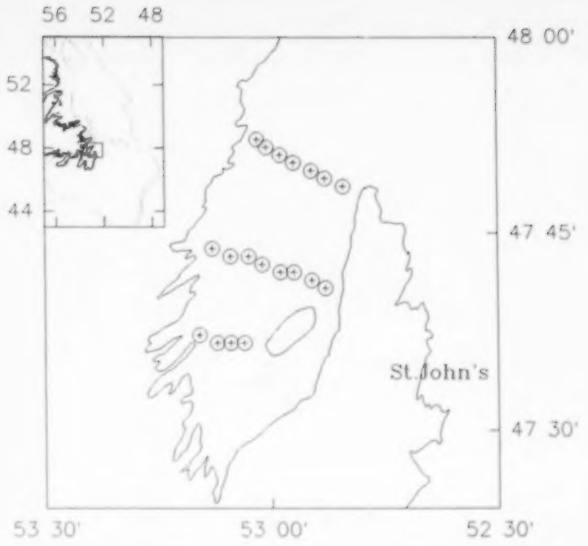

FIG. 1. Map of Conception Bay, Newfoundland, Canada, showing the station locations occupied as part of the survey.

Conception Bay was chosen as the study site partly because it is an area of significant egg production for many species (P. Pepin, unpublished data) and it may serve as a nursery area for some commercially important stocks (e.g., *Gadus morhua* (Templeman 1981)). The physical characteristics of the bay allowed frequent and complete surveys of the system to be conducted during a short study period. It is also possible to monitor short-term variations in current regime (J. Helbig and P. Pepin, unpublished data). These features combined to provide a model system that can readily be monitored on time and space scales important to the study of animals with high turnover rates.

Sampling Procedure

Nineteen stations were sampled in Conception Bay from June 25 to July 16, 1990, from a 22-m vessel (CSS *Shamook*). Stations were spaced 2–5 km apart along three major transects approximately 15 km apart (Fig. 1). In general, nine or 10 stations were occupied on a single day. Six complete surveys of Conception Bay were conducted during the study. Mechanical and weather problems prevented continuous coverage on six days during the 3-wk period. The survey was designed to provide thorough coverage of all water masses which fish larvae may occupy. The transects were oriented east to west because of variations in temperature and salinity caused by coastal upwelling that may result in variations in biotic environment associated with larvae (Frank and Leggett 1983). Transects were situated at the mouth, center, and bottom of the bay to ensure coverage of areas that may represent shelf, mixed, and coastal biological provinces within this system.

Ichthyoplankton samples were obtained during daylight hours (07:00–19:00) using a 4-m^2 Tucker trawl equipped with 1000-, 570-, and 333-μm-mesh nitex. At each station, a single oblique tow of approximately 15 min was made at 2 kn. The net was lowered at a rate of 0.25 $m \cdot s^{-1}$ to 40 m and retrieved at a rate of 0.064 $m \cdot s^{-1}$. Maximum tow depth was chosen to encompass the mixed layer in which most of the larval fish occur (Kendall and

TABLE 1. Species, length interval, mortality rate, number of specimens captured (n), and significance of the estimated mortality rate for the analysis performed using the traditional length-based method (Ahlstrom, 1954; Smith and Richardson 1977).

Species	Length interval (mm)	Mortality rate (mm^{-1})	n	Significance level
Aspidophoroides monopterygius	11–25	0.12	32	<0.01
Clupea harengus	10–17	0.13	156	<0.05
Gadus morhua	5–18	0.34	995	<0.001
Glyptocephalus cynoglossus	4–6	1.03	29	0.47
Hippoglossoides platessoides	5–17	0.66	13886	<0.001
Liparis sp.	4–7	0.97	90	<0.01
Liparis atlanticus	3–8	0.71	866	<0.05
Liparis gibbus	5–16	0.22	60	<0.01
Mallotus villosus	6–13	1.30	29483	<0.001
Pleuronectes americanus	3–7	1.41	694	<0.01
Pleuronectes ferrugineus	2–7	1.43	3409	<0.05
Scomber scombrus	2–3	1.90	17	0.34
Sebastes sp.	8–10	0.94	264	0.20
Stichaeus punctatus	12–26	0.36	307	<0.001
Tautogolabrus adspersus	2–3	2.43	54	0.53
Ulvaria subbifurcata	6–15	0.82	8861	<0.001

Naplin 1981; Boehlert et al. 1985; Lenarz et al. 1991; J. Anderson, Northwest Atlantic Fisheries Center, St. John's, Nfld., personal communication). Volumes filtered were estimated from the average of two General Oceanics flowmeters positioned at the mouth of the net. On deck, the net was washed and the samples were preserved in 2% buffered formaldehyde.

Laboratory Analysis

Fish eggs and larvae were sorted and identified to species or the lowest taxonomic level possible by the Atlantic Reference Centre (Huntsman Marine Science Centre, St. Andrews, N.B.). Subsampling of an individual taxon was performed when a species exceeded 200 individuals per stage (i.e., eggs or larvae) in a sample. A beaker technique (van Guelpen et al. 1982) was used to subsample. The length frequency distribution for each species and sample was estimated by measuring up to 200 larvae of a species per sample. Larvae were measured to the nearest millimetre for standard length using a dissecting microscope and a gridded background. Abundance (number per 1000 m^3) was calculated for each species for every 1-mm length interval for each sample. Abundance estimates were corrected for the number of larvae that could not be measured due to damage (<5%) or sampling. The length frequency distribution for each species and day was calculated by summing estimated abundances for each length interval of each species over all stations with positive catches and dividing by the total number of stations sampled on that day. The cumulative length frequency distribution (i.e., total production) for the survey period was estimated by summing each day's length frequency distribution.

No correction was made to the estimates of abundance for possible size-dependent changes in catchability. Failure to correct for such variations may result in a positive bias in estimated mortality rates. Morse (1989) corrected estimates of abundance based on diurnal changes in catchabilities for each species. I compared Morse's corrected estimates of Z with those I gathered from other sources (Pepin 1991), which were largely uncorrected for changes in catchability. In my review (Pepin 1991), I found that mortality estimates that were not corrected for variations in catchability were positively biased relative to Morse's (1989) data. However, I found no significant differences in the slopes of the size-dependent mortality functions estimated from Morse's (1989) study or my review (Pepin 1991). Because the objective of this study is to assess the accuracy of the size-dependent mortality hypothesis, possible variations should not influence the trend of this relationship.

Estimates of Larval Fish Mortality Rates

The traditional length-based method of estimating larval fish mortality rates was described by Ahlstrom (1954) and Smith and Richardson (1977). The mortality rate (per millimetre) is estimated using the survival curve (i.e., the descending limb of the cumulative length frequency distribution) of larvae such that

$$(1) \quad N = ae^{-ZL}$$

where N is the abundance of larvae (number per 1000 m^3), L is the length (millimetres), Z is the mortality rate (per millimetre), and a is a constant. An estimate of Z is obtained using linear regression of a log-transformation of equation 1. In order to apply the length-based method correctly, it is important to have a representative sample of the population and that the production rate of the youngest larvae be constant (i.e., the sample must represent a stable age distribution). In addition, one assumes that immigration and emigration are negligible. Implicit in this method is the assumption that size is a good approximation of biological age (i.e., constant growth rates). Mortality rates (per millimetre) estimated using equation 1 are often converted to instantaneous daily rates using deterministic estimates of growth (millimetres per day) obtained from modal analysis of lengths or predictive models (e.g., regressions) fitted to length/otolith increment data. I did not carry out such a conversion. To properly convert length to age, there must be some consideration of variations in size about an age, as obtained using an age–length key (Somerton and Kobayashi 1992). Residuals were assessed

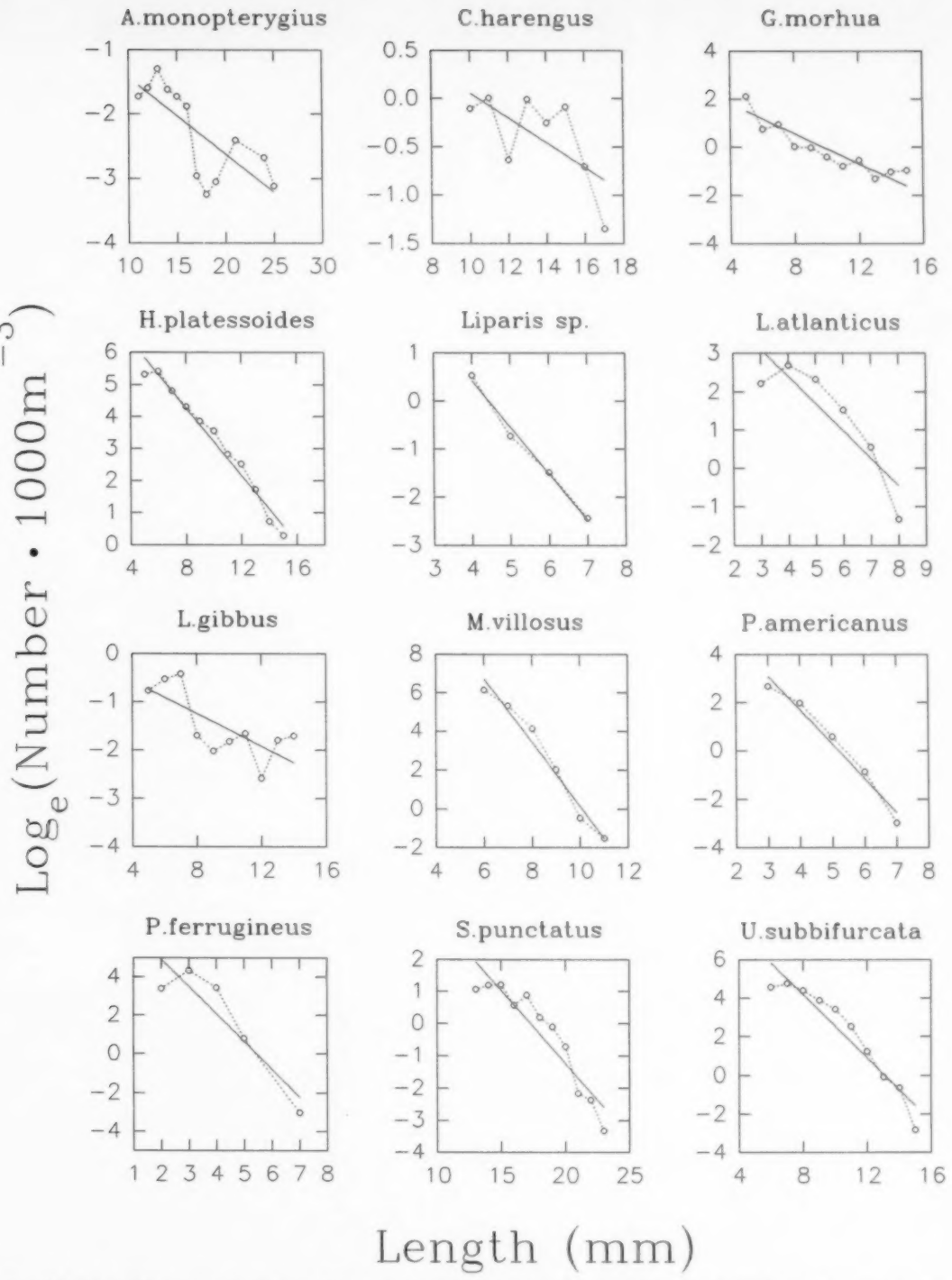

FIG. 2. Cumulative length frequency distribution of 12 species caught during this survey for which it was possible to estimate mortality rates using Ahlstrom's (1954) length-based method (Table 1). The open symbols and broken lines show the average cumulative abundance of fish larvae as a function of length class. The slope of the solid line represents the estimated mortality rate (equation 1).

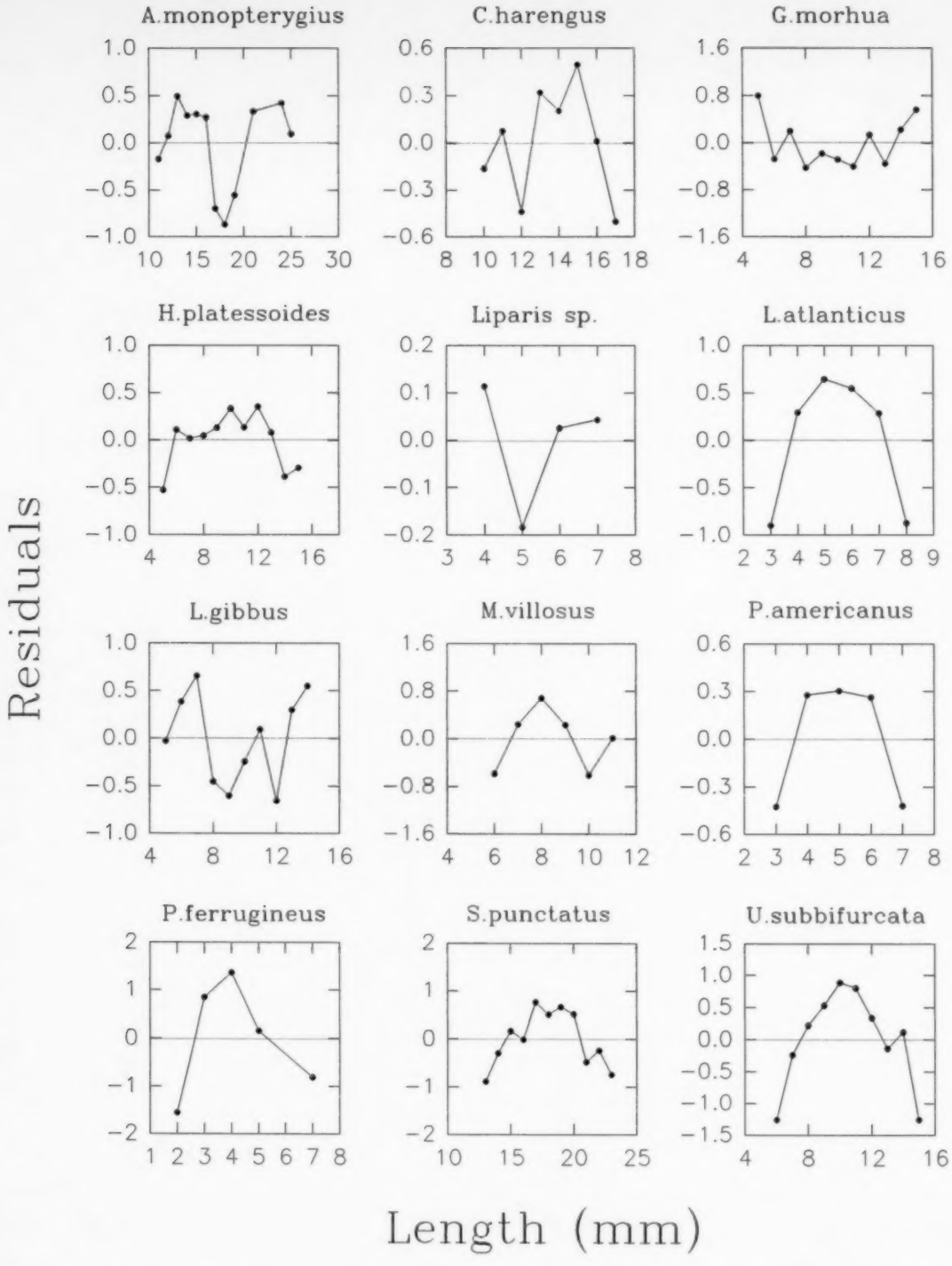

FIG. 3. Residual plots of the relationship fitted to estimate mortality rates (equation 1) for the 12 species for which significant estimates of larval fish mortality rates were obtained (solid line in Fig. 2).

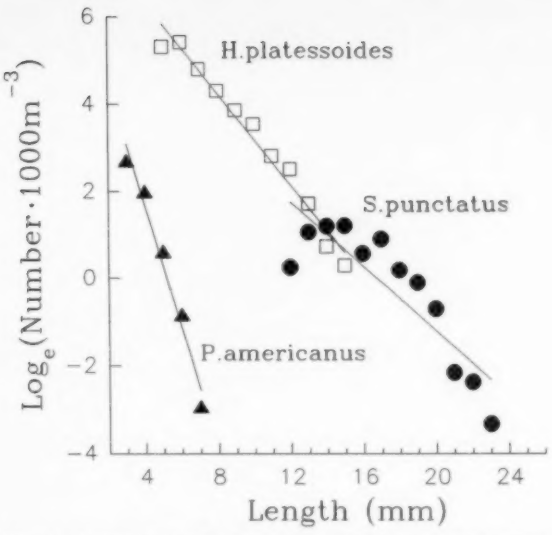

FIG. 4. Cumulative length frequency distribution of three dominant species sampled during the study period and the regression lines which describe the mortality rates estimated using the length-based method (equation 1).

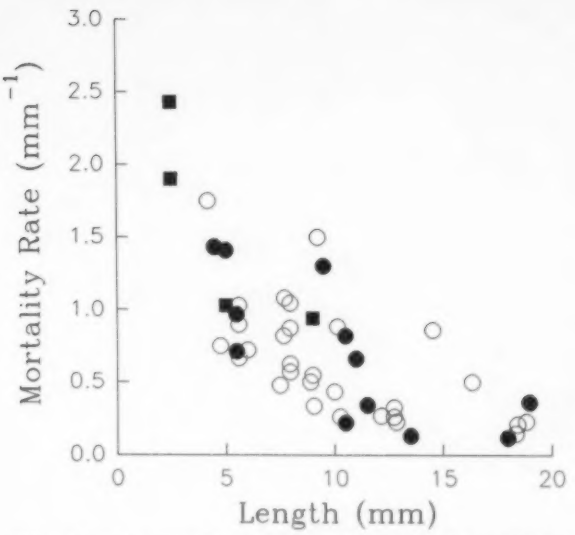

FIG. 5. Mortality rates of larval fish from this study (solid symbols) and from Pepin's (1991) summary of the literature (open symbols) in relation to the midpoint of the length interval over which mortality rates of the organisms were estimated. Circles represent values that are significantly different from zero whereas squares represent values that are not significantly different from zero (Table 1).

for randomness using a runs test based on the sign of the residual deviation from the fitted model (equation 1) (Sokal and Rohlf 1981, p. 782–787).

Results

Sixteen taxa of fish larvae were captured during the survey period (Table 1). Peak mean density ranged from slightly less than 0.1 individual·1000m^{-3} up to over 475 larvae·1000 m^{-3}. The cumulative length frequency distributions for the 12 most abundant species (Fig. 2) show a pattern which is not inconsistent with that of a closed population with stable age distribution. In the cases of *A. monopterygius*, *C. harengus*, and *L. gibbus*, there is some indication of multiple cohorts in the survey region but the separation between them is generally weak. In all instances the pattern of abundances does not indicate that larger individuals are immigrating into the survey area. Although there was evidence of variations in the abundance of eggs and yolk-sac larvae (R. Laprise and P. Pepin, in preparation), these fluctuations were sufficiently small that production was treated as constant for this study. Mortality rates (per millimetre) estimated using equation 1 were significantly different from 0 for 12 of the 16 species studied (Table 1; Fig. 2). The log-linear model (equation 1) appears to provide a good general relationship describing the length-based survival curve (average r^2 = 0.82, range 0.45–0.98). Results not significantly different from 0 were generally associated with small sample sizes or a limited range of size categories.

Residuals from equation 1 do not show strong evidence of a lack of fit to the model (Fig. 3). Although seven of the 12 species appear to show some evidence of a dome-shaped pattern in the residuals, the exceptions being *A. monopterygius*, *C. harengus*, *G. morhua*, *L. gibbus*, and *M. villosus*, a runs test showed no significant departures from randomness for any species. Combining probabilities from the runs test among species (Sokal and Rohlf 1981, p. 779–782) did not show any significant pattern in the residuals. Thus, a model of constant mortality during the larval phase appears to be appropriate for length-based estimates of mortality. This is most apparent when several species are plotted on the same graph (Fig. 4). It is evident that the rate of decrease, in relation to size, is nearly constant for those species. However, the rate of decrease appears to be influenced by the size range over which the species is sampled.

Comparison with Other Studies

Mortality rates estimated from this survey were compared with those studies summarized in an earlier review (Pepin 1991). The latter represents information gathered from 17 species sampled on a wide range of space and time scales. All estimates of larval fish mortality rates from the survey of Conception Bay, whether significant or not, lie within the range of estimates observed in other studies in a comparison among species (Fig. 5). The functional relationship for all the data obtained in this survey is

$$(2) \quad Z = 7.7L^{-1.20}$$

($r^2 = 0.67$, $p < 0.0001$, $n = 16$) where Z is the mortality rate (per millimetre), L is the midpoint of the length frequency distribution of each taxon (millimetres), and the standard error of the exponent is 0.21. Equation 2 is not significantly different ($F_{1,45} < 0.0001$, $p = 0.97$) from the relationship estimated by Pepin (1991) ($Z = 8.5L^{-1.27}$, $r^2 = 0.59$, $p < 0.001$, $n = 32$) from a review of the literature. The estimated mortality rates derived from this survey appear to be consistent with those from other environments and species, suggesting that results of this study provide a realistic representation of mortality rates estimated using a length-based method that uses size as a proxy for

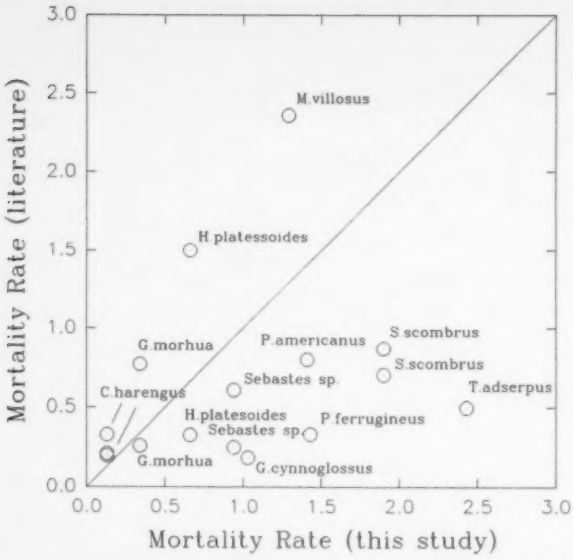

FIG. 6. Mortality rates observed in other studies for individual species plotted in relation to the estimates obtained in this survey. Data from other studies were collated from Pepin's (1991) review for *C. harengus* (2), *G. morhua*, *H. platessoides*, *P. americanus*, *M. villosus*, *S. scombrus*, and *Sebastes* sp. and from Morse's (1989) analysis for *C. harengus*, *G. morhua*, *G. cynoglossus*, *H. platessoides*, *P. ferrugineus*, *S. scombrus*, *Sebastes* sp., and *T. adspersus*. The one-to-one line is shown for reference.

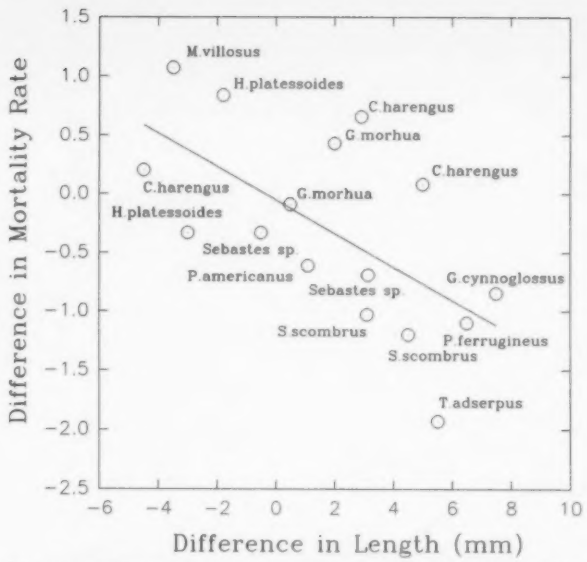

FIG. 7. Difference between mortality rates for individual species observed in other studies with those estimated in this survey ($Z_{LIT} - Z_{CB}$ where Z are the mortality rates and the subscripts LIT and CB refer to literature values and those obtained from this survey of Conception Bay, respectively) plotted in relation to the difference in the midpoint of the range in lengths sampled in each study ($L_{LIT} - L_{CB}$ where L are the lengths and the subscripts are as described above). A positive value on the y-axis indicates that the estimated mortality rate in the other study was greater than that observed in this study. A negative value on the x-axis indicates that larvae were smaller in the other study. See the caption to Fig. 6 for details about sources.

biological age (Ahlstrom 1954; Smith and Richardson 1977).

Although the general relationship of mortality in relation to size of larvae is consistent among species and studies, there appears to be little correspondence in mortality rates within species when comparing this and other studies (Fig. 6). Although mortality rates estimated for an individual species in this survey tend to be higher that those observed in previous studies (10:5), the range of observations about the one-to-one line is about equal. However, the difference in mortality rates among surveys within species is strongly correlated with the difference in size, or size range, of larvae sampled during each survey ($r = 0.68$, $P < 0.01$, $n = 16$) (Fig. 7). This indicates that as the size range of larvae sampled in each survey increases, the estimated mean mortality rate for each species decreases.

Discussion

The results of this study, and a comparison with previous observations, raise some important questions about the validity and generality of the size-dependent mortality hypothesis for larval fish. In a comparison among species, the results from this survey are consistent with previous reviews that indicate that as the size of fish larvae increases, the observed mortality rate decreases (Fig. 5). However, a close examination of each species sampled as part of this study clearly shows that the rate of decrease in numbers, in relation to size, does not significantly depart from that of a constant exponential decay (Fig. 2 and 3). One might conclude that species which produce large larvae will experience low but constant mortality rates during this stage of the life cycle relative to a species which produces small larvae. Such an interpretation may appear reasonable but a comparison of results for individual species among different sources clearly shows that estimates of mortality rates are strongly associated with the range of size sampled during specific surveys (Fig. 6 and 7). The change in estimated mortality rates in response to variations in the size categories sampled in each survey raises questions about the accuracy and validity of length-based estimates of larval fish mortality rates. Given that the general hypothesis of size-dependent mortality of fish larvae is founded largely on length-based estimates, the findings of this study potentially discredit the interpretation that the size-dependent mortality hypothesis is only valid for a general comparison of life history strategies among species. At the same time, the change in estimated mortality rate in relation to the change in length interval sampled among surveys of a single species is inconsistent with the evidence that Ahlstrom's (1954) length-based model provides a reasonable representation of the rate of loss of fish larvae with increasing size (Fig. 2 and 3). Since it is also apparent that the size-dependent exponential decay model has also been considered appropriate within other studies (e.g., Lenarz 1973; Morse 1989), it appears that length-based estimates of larval fish mortality rates are potentially biased and inaccurate. Consequently, a general size-dependent mortality hypothesis for larval fish (e.g., Peterson and Wroblewski 1984; McGurk 1986; Pepin 1991) cannot be supported by the information from which it is derived. The observed pattern of differences in mortality rates of fish larvae among species and within species among surveys can be caused by (1) unaccounted variations in catchability, (2) age-related variations in mortality, or (3) failure in some assumptions of length-based methods of estimation.

One might argue that differential avoidance or behaviour may limit the ability to accurately estimate and compare mortality rates of different species of fish larvae. However, if avoidance is size-dependent (e.g., Murphy and Clutter 1972; Brander and Thompson 1989; Morse 1989; McGurk 1992), then its effect should be apparent in the results obtained both among and within species. Avoidance by larger sized larvae would result in an apparent underestimate of their abundance. This would translate into an apparent increased rate of loss as the range of sizes sampled is increased. The general mortality–size relationship would then be opposite to that proposed in previous reviews (Peterson and Wroblewski 1984; McGurk 1986: Pepin 1991) if avoidance significantly biased the estimated mortality rates. Therefore, gear avoidance cannot account for the apparent size-dependent pattern of mortality rates.

A fundamental problem with the traditional method of estimating mortality rates may arise from the general assumption that the size of fish larvae is a good proxy for their age. In the traditional length-based method (Ahlstrom 1954), size forms the basis of the frequency distribution of catches. Implicit in this model is the consideration that size is a good approximation for age. Often, mortality rates calculated in units of per millimetre are converted to instantaneous daily mortality rates (millimetres per day), estimated from a general functional relationship. This could be reasonable if growth rates were constant throughout the population. However, there is clear evidence that within a cohort, variations in growth rate can be substantial (Rosenberg and Haugen 1982; Methot 1983; Penney and Evans 1985). The consequence of this variability is that as the larvae age, the variance in the length frequency distribution for larvae of a given age increases (Chambers et al. 1988). As a result, analyses which use size to represent age are likely to obtain biased estimates of mortality rates. The estimated average mortality rate is likely to change as older and more variable sized larvae are included in the length frequency distribution, independently of whether mortality is influenced by the size of the animals. A comparison of mortality rates estimated from different ranges of age and/or size may lead to inaccurate conclusions about factors that influence their variability. A solution to this problem would be to obtain an accurate age–length key for the sample population. Previous studies of larval fish age–length relationships have generally provided general linear models (i.e., regression of length in relation to age). More detailed distributional information af age could be more appropriate in reducing the potential bias of the traditional method used to estimate larval fish mortality rates. A proper and accurate age–length key would provide the appropriate representation of the age frequency distribution of the population relative to that provided by a length frequency distribution which assumes a constant and uniform growth (Somerton and Kobayashi 1992). By using the combination of age and size, we could gain a more realistic understanding of the factors that may influence early life history survival because processes are occurring through time, not length. This could enable us to assess whether significant age-dependent variations in survival exist (e.g., Litvak and Leggett 1992) or whether variations in length-at-age could have biased previous estimates of larval fish mortality rates.

Essig and Cole (1986) presented two approaches to estimating larval fish mortality rates from aged specimens. Both their proposals use the descending limb of an increment frequency distribution, but neither provides the basis for developing a proper age–length key which is required to properly assess size dependency. Instead, Essig and Cole (1986) suggested the development of a generalized growth curve which assumes that the variation in size-at-age is constant, which may not be correct (e.g., Chambers et al. 1988). The potential importance of size dependency has been acknowledged in Lo's (1985, 1986) generalized Pareto model of early life history mortality. However, as with other efforts to provide age-based estimates of mortality, Lo's (1985, 1986) model is based on a generalized growth curve that may not entirely reflect the extent of variation in size-at-age. Increased variation in size with age (Kramer and Zweifel 1970; Methot and Kramer 1979; Butler 1989) could result in an apparently larger number of older larvae when a generalized growth curve is used to convert length to age. The resulting pattern of numbers-at-age would then be consistent with a model based on size-dependent mortality (e.g., Pareto model). Any variations in the growth rate function could potentially bias estimated mortality rates (Lo et al. 1989). An age–length key could reduce the possibility of bias because of assumptions in the mean and variance about a growth function.

The consequences of this study are that surveys of fish larvae need to provide proper sampling of the age frequency distribution of the population in order to accurately estimate mortality rates. Because previous measurements of early life history vital rates may not be accurate reflections of the true variability in mortality, the general size-dependent model governing the study of larval fish may need to be evaluated afresh. It has been suggested that size-dependent mortality is largely the result of changing vulnerability of larvae to predators. The consistency between this latter concept and the association between larval fish mortality rates and their size (Peterson and Wroblewski 1984; McGurk 1986; Pepin 1991) suggests that our understanding of the dynamics of marine systems is accurate (e.g., Peterson and Wroblewski 1984). Evidence presented in this study casts some doubt not only on our measurements of larval fish mortality but also on some concepts dealing with the factors that may determine survivorship and recruitment. Processes that influence both development and losses of larval fish show size dependency under specific laboratory conditions (Miller et al. 1988; Bailey and Houde 1989; Fuiman 1989; MacKenzie et al. 1990; Pepin et al. 1992). There are also instances where size-dependent processes are operating in the field (e.g., Post and Evans 1989). Whether the overall effect of size-dependent processes on larval fish mortality leads to a pattern consistent with the general theory outlined above has yet to be determined. It is possible that within marine ecosystems, differences in mortality rates among taxa or trophic levels may show a general trend towards size dependency (Peterson and Wroblewski 1984; McGurk 1986) but such trends may not be followed within specific levels of organization. To resolve the potential significance of size-dependent processes to early life history survival, it is essential that the effects of potentially confounding factors (e.g., age) be taken into consideration. Although there may be a suite of physiological factors influencing the susceptibility of larvae to die (e.g., condition, experience, genetics), information on the distribution of size and age are the only elements that can potentially be used in backcalculating earlier states of the individuals and the population. Proper sampling of the age composition through time is the only manner by which estimates of the change in abundance (i.e., mortality) can be obtained. Because of the variation in length-at-age, length-based methods of studying variations in mortality rates appear to be inappropriate.

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Use of Parasites for Separating Stocks of Greenland Halibut (*Reinhardtius hippoglossoides*) in the Canadian Northwest Atlantic

J.R. Arthur and E. Albert

Department of Fisheries and Oceans, Maurice Lamontagne Institute, P.O. Box 1000, Mont-Joli, QC G5H 3Z4, Canada

Arthur, J.R., and E. Albert. 1993. Use of parasites for separating stocks of Greenland halibut (*Reinhardtius hippoglossoides*) in the Canadian northwest Atlantic. Can. J. Fish. Aquat. Sci. 50: 2175–2181.

The usefulness of parasites as biological tags for stocks of Greenland halibut (*Reinhardtius hippoglossoides*) occurring off the Atlantic coast of Canada and in the Gulf of St. Lawrence was investigated. Nonparametric discriminant function analyses of eight collections comprising a total of 231 large fish (>39 cm) using counts for selected parasite taxa (*Corynosoma strumosum* juvenile, *Otodistomum* sp. metacercaria, Contracaecinea spp. larva, *Anisakis simplex* larva, and *Pseudoterranova decipiens* larva) gave accurate results (almost 100% correct classification) for the separation of fish from the Gulf of St. Lawrence from those collected from adjacent areas of the Saguenay Fjord and the Atlantic Ocean off Labrador. Little, if any, mixing of subadult or adult fish apparently occurs among these areas.

Une étude a été menée afin de déterminer l'utilité des parasites comme étiquettes biologiques pour l'identification des stocks de flétan du Groënland (*Reinhardtius hippoglossoides*) de la côte atlantique et du golfe du Saint-Laurent. L'échantillonnage de 231 poissons de taille supérieure à 39 cm a été éffectué à huit sites différents. Des analyses discriminantes non paramétriques utilisant cinq taxons (la forme juvénile de *Corynosoma strumosum*, le stade métacercaire d'*Otodistomum* sp. et le stade larvaire de Contracaecinea spp., d'*Anisakis simplex* et de *Pseudoterranova decipiens)* ont réussi à séparer de façon très précise les poissons du golfe du Saint-Laurent de ceux des régions adjacentes du fjord du Saguenay et de l'océan Atlantique au large du Labrador. Presque 100 % des poissons ont été reclassifiés correctement dans leur région d'origine. Il semble y avoir très peu ou même pas de migration des flétans du Groënland immatures ou adultes entre ces régions.

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The Greenland halibut (*Reinhardtius hippoglossoides*) is a deepwater flatfish occurring in northern waters of the Atlantic and Pacific oceans. In the northwestern Atlantic Ocean, it is distributed from arctic waters southward off Labrador and Newfoundland to Georges Bank and into the Gulf of St. Lawrence (Scott and Scott 1988). It is of high commercial importance to Canada's Atlantic fisheries, with an estimated 1991 landing of almost 75 000 t for the Newfoundland/Labrador fishery (NAFO subarea 2 and divisions 3KL) (see Brodie and Baird 1992). In 1987, this species was the second most valuable groundfish in the Gulf of St. Lawrence, with landings of about 11 000 t, although recent catches have declined sharply to an estimated 2200 t in 1991 (B. Morin, Maurice Lamontagne Institute, Mont-Joli, Que., personal communication).

The current concept of stock structure, based on morphometric, meristic, biochemical genetic analysis, and parasitological evidence, is that a single stock exists for Canadian waters from the Grand Bank to Davis Strait. A discrete spawning is believed to occur in the Gulf of St. Lawrence with an unknown amount of recruitment into this stock from the larger northwest Atlantic stock (see Templeman 1970; Fairbairn 1981; Tremblay and Axelsen 1981; Bowering 1982, 1983, 1988; Khan et al. 1982; Misra and Bowering 1984; Bowering and Chumakov 1989; Riget et al. 1992). Greenland halibut occurring in the Saguenay Fjord, a deepwater fjord entering the estuary of the St. Lawrence River, may constitute an isolated population, although to date this hypothesis has not been investigated.

Bowering (1982) suggested that significant recruitment of fish to the stock in the Gulf of St. Lawrence comes from the larger population of Greenland halibut occurring off Labrador. He based this conclusion on similar peaks in annual abundance and observations on maturity rates. Young stages (eggs, larvae, and/or juveniles) are believed to migrate or to be carried by prevailing currents into the Gulf via the Strait of Belle Isle (see Fig. 1). The absence of larger Greenland halibut in the Gulf (i.e., males >60 cm and females >70 cm) was considered likely due to their emigration upon approaching maturity. It was hypothesized that these fish may undergo a unidirectional migration, passing outside the Gulf via the Cabot Strait to participate in spawning in the Davis Strait with the larger northwest Atlantic stock. The occurrence of high concentrations of fish in an advanced stage of maturation in the northwestern part of the Gulf of St. Lawrence in late autumn and the presence of mature individuals at the end of November, as well as conditions that are conducive to successful reproduction, led Tremblay and Axelsen (1981) to believe that a significant amount of spawning occurs in the Gulf. Similarly, Bowering (1983) concluded that Greenland halibut occurring in the Gulf of St. Lawrence appeared to be composed of a small stock which spawns there supplemented by immigrants from the Labrador region which may undergo a spawning migration outside the Gulf.

Parasites have been widely used as "biological tags" to

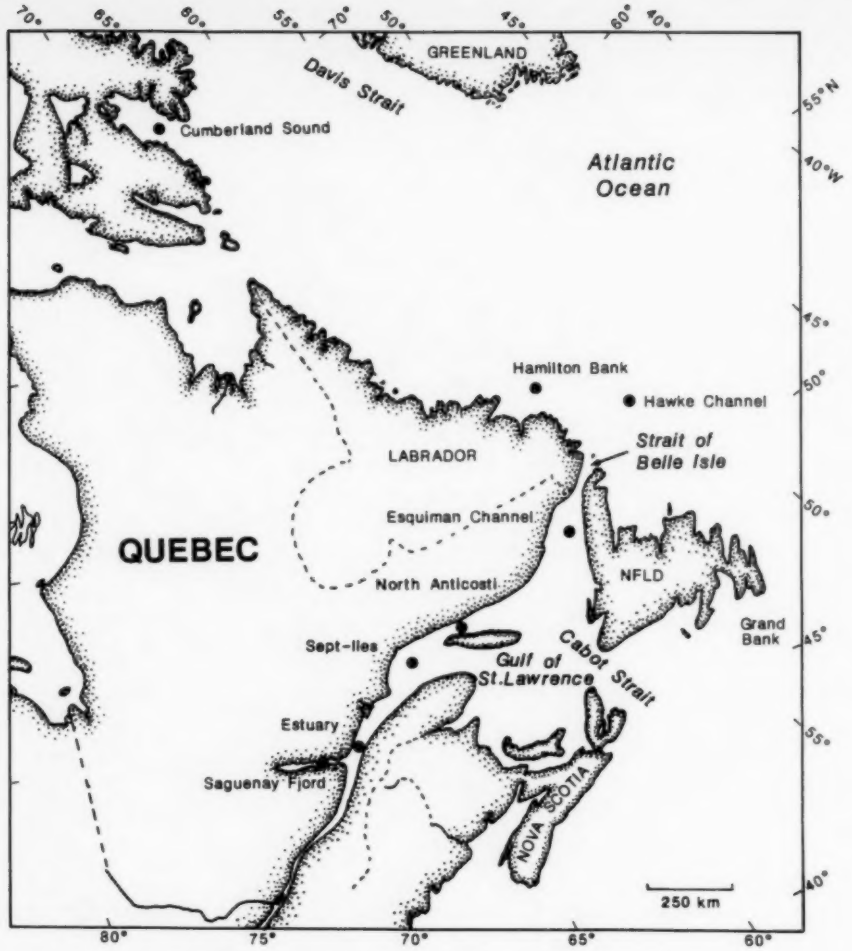

FIG. 1. Map of the northwest Atlantic Ocean off Canada showing collection localities.

provide information for fisheries management on the movements and population discreteness of their fish hosts (see MacKenzie 1983; Lester 1990; Moser 1991; Williams et al. 1992). Only two studies have attempted to separate stocks of Greenland halibut using their parasites. In a study of the blood protozoans of this fish in Canadian Atlantic waters, Khan et al. (1982) found geographical variations in the prevalences of two species (*Haemohormidium terranovae* So, 1972 and *Trypanosoma murmanensis* Nikitin, 1927) which led them to postulate that separate stocks occurred in the Davis Strait, off southern Labrador/northern Newfoundland (probably a cline between areas), in the Gulf of St. Lawrence, and at Fortune Bay, southern Newfoundland. Recently, Krzykawski and Wierzbicka (1992) used differences in growth rates and morphometric, meristic, and parasitological data to show that fish occurring in the Barents Sea and off Labrador belong to different stocks.

The present study was undertaken to determine if multivariate analyses based on data for some of the more than 40 parasite species which we have identified from Greenland halibut in Canadian Atlantic waters (J.R. Arthur and E. Albert, unpublished data) could provide more detailed information on the discreteness of stocks of this fish.

Materials and Methods

Host and Parasite Collections

Preliminary investigations showed that small Greenland halibut (<20 cm) were too lightly parasitized, both in prevalences and intensities of infections, for parasites to be useful as stock separators (J.R. Arthur and E. Albert, unpublished data); thus, only large fish were used for this study. A total of 231 fish of size ≥39 cm were examined from eight areas off the Atlantic coast of Canada. These included one sample of fish collected from Cumberland Sound by long line, two from off Labrador (Hamilton Bank and Hawke Channel), and four from the Gulf of St. Lawrence (Estuary, Sept-Îles, North Anticosti, and Esquiman Channel) collected by trawl and one from the Saguenay Fjord obtained from the winter sport fishery (Fig. 1). Fish were measured and individually bagged and deep-frozen immediately after capture for later laboratory examination. As all fish from

TABLE 1. Summary of data for collections of Greenland halibut.

Area	Latitude (N)	Longitude (W)	Date (d/mo/yr)	Depth (m)	*N*	Total length (mm)[a]	Age (yr)[a]	Sex ratio (m:f)
Cumberland Sound	65°47′	66°05′	-/01/90	500	30	688.4 ± 101.1 (492–872)	11.2 ± 1.9 (8–15)	0:30
Hamilton Bank	54°25′	56°08′	11/11/90	440	30	447.0 ± 45.9 (400–560)	7.5 ± 0.9 (6–10)	12:18
Hawke Channel	53°02′	52°44′	5/11/90	470	30	448.0 ± 35.1 (410–550)	7.6 ± 0.9 (6–10)	11:19
Esquiman Channel	49°25′–50°12′	57°45′–59°08′	24–26/08/90	143–320	30	537.1 ± 62.8 (416–659)	8.5 ± 1.4 (6–12)	9:21
North Anticosti	49°36′–49°48′	61°28′–62°54′	3–26/09/90	179–279	30	494.1 ± 58.1 (412–619)	7.7 ± 1.0 (6–10)	9:21
Sept-Îles	49°34′–50°00′	65°05′–66°20′	5–9/09/90	202–361	30	473.0 ± 59.9 (390–650)	7.1 ± 1.0 (6–10)	18:12
Estuary	48°40′–48°49′	68°07′–68°38′	11–12/09/90	210–350	21	531.0 ± 71.1 (410–660)	8.5 ± 1.3 (7–11)	2:19
Saguenay Fjord	48°15′–48°25′	70°11′–70°51′	31/01–10/03/91	85–150	30	487.5 ± 44.0 (413–555)	7.4 ± 0.9 (6–9)	8:22

[a]Mean ± *SD (range in parentheses).*

Cumberland Sound and the Saguenay Fjord were received frozen, estimates of fresh length were made for these fish using a regression of fresh length onto frozen length obtained from fish from Sept-Îles. In the few cases where age could not be determined from saccular otoliths, ages were estimated from age/length regressions for the individual collections involved. Host collection data are summarized in Table 1, and collection localities are shown in Fig. 1.

Complete examinations for protozoan and metazoan parasites were performed using standard parasitological methods. Fish were thawed in the laboratory and the external surface rinsed. The rinse was then collected, settled, and the sediment examined for ectoparasites using a stereomicroscope. Fish were measured (total length) and weighed. The gills were removed, rinsed, and the arches examined individually. The buccal cavity was rinsed and the opercula, eyes, and rinse water examined separately. Saccular otoliths were removed for subsequent age determination. The fins were removed, skinned, and individually examined for encysted parasites. The internal organs (heart, liver, spleen, gall bladder, digestive tract, gonads, kidney, urinary bladder) were inspected for parasites free or encapsulated on the exterior, separated, and then individually examined. The stomach, pyloric caeca, and intestine were separated, opened longitudinally, and their contents rinsed into beakers where they were mixed with sodium bicarbonate and settled to remove endoparasitic helminths. The walls of the stomach, pyloric caeca, and intestine, and the liver, spleen, kidney, and heart were compressed between glass plates and examined for parasites. The body cavity was rinsed and the rinse collected and examined. Squash preparations made from liver, spleen, kidney, gonads, intestine, muscle, and brain tissue and scrapings from the urinary and gall bladders were examined for Protozoa using a compound microscope at a magnification of 400×. Preparations not found to harbour protozoans after 5 min of examination were considered uninfected. The body musculature was removed from the vertebral column, the skin removed from the fillets, and the fillets and flaps thinly sliced and inspected for helminths and protozoan cysts.

All parasites were sorted into major taxonomic groups, cleaned, counted for each organ (metazoans only), and fixed in 10% buffered formalin (Protozoa), 70% ethanol with 10% glycerin (Nematoda, Crustacea), or alcohol – formalin – acetic acid (AFA) (platyhelminths and Acanthocephala) for later identification to lowest possible taxon. Digenea, Cestoda, and Acanthocephala were stained with Delafield's hematoxylin or Schneider's acetocarmine and mounted in Permount. Nematoda and Acanthocephala were routinely cleared by evaporation in glycerine in 70% ethanol and examined as temporary mounts in glycerine. Copepoda were dissected and appendages examined as temporary mounts. All unmounted metazoan parasites were transferred to 10% glycerine in 70% ethanol for long-term storage.

Parasites for use as possible biological tags for Greenland halibut were chosen based on three criteria: (1) that infections be of relatively long duration (years rather than months), (2) that no parasite reproduction occur on or in the host, and (3) that the parasite be relatively abundant in at least one of the eight collections examined. Of the more than 40 parasites identified (J.R. Arthur and E. Albert, unpublished data), 14 were considered potentially useful as long-term biological tags and five met all three criteria. These included one digenean metacercaria (*Otodistomum* sp.), three larval nematodes (*Anisakis simplex* (Rudolphi, 1809), *Pseudoterranova decipiens* (Krabbe, 1878), and Contracaecinea spp.), and one juvenile acanthocephalan (*Corynosoma strumosum* (Rudolphi, 1802)).

Statistical Analyses

Summary statistics for the five parasite taxa (Table 2) include prevalence (percent infected) and intensity of infection (number of parasites per infected fish), expressed as the mean plus and minus the standard deviation, followed by the range as recommended by Margolis et al. (1982).

Since parasite counts for all areas were not normally distributed and normality could not be achieved using various transformations, nonparametric discriminant analyses (SAS version 6, SAS Institute, Inc. 1989) were used to investigate the usefulness of parasites in separating host collections. As all parasites used in this study are relatively long lived and are thus accumulated with host age (length), trends due to associations between parasite numbers and fish length were removed by adjusting counts for fish length. For each parasite species from each area, a regression of parasite numbers on fish length was undertaken (SAS GLM procedure, SAS Institute, Inc. 1989) and the resulting relationship used to adjust the parasite number to that expected for a fish of 513 mm, which is the overall average host length for the eight collections. After this adjustment, regressions did not show any association between corrected parasite numbers and fish length, weight, or sex. Nonparametric discriminant analysis was conducted using the normal kernel density estimation with an R value of 1.0. Cross validation was used to estimate the accuracy of classification rules. With this technique, each fish is removed from the "training" data set, the classification rule is recalculated, and the classification of the omitted fish is estimated.

TABLE 2. Data summary for parasites used to separate collections of Greenland halibut.

Parasite	Cumberland Sound ($n = 30$)		Hamilton Bank ($n = 30$)		Hawke Channel ($n = 30$)		Esquiman Channel ($n = 30$)	
	P(%)[a]	Intensity (range)[b]	P(%)	Intensity (range)	P(%)	Intensity (range)	P(%)	Intensity (range)
Anisakis simplex (l)[c]	10.0	1.0 ± 0.0 (1)	40.0	1.6 ± 0.8 (1–3)	66.7	2.2 ± 1.5 (1–6)	100.0	7.6 ± 5.1 (1–26)
Corynosoma strumosum (j)	30.0	1.6 ± 0.7 (1–3)	26.7	1.1 ± 0.4 (1–2)	0.0	—	3.3	2.2 ± 0.0 (2)
Pseudoterranova decipiens (l)	0.0	—	3.3	1.0 ± 0.0 (1)	0.0	—	10.0	1.7 ± 0.6 (1–2)
Otodistomum sp. (m)	0.0	—	0.0	—	0.0	—	66.7	21.9 ± 23.8 (1–68)
Contracaecinea (l)	100.0	12.4 ± 9.5 (2–37)	96.7	6.4 ± 4.4 (1–21)	96.7	16.7 ± 11.9 (5–50)	86.7	6.4 ± 6.3 (1–28)

[a]Prevalence (percent infected).
[b]Intensity (mean ± SD) followed by range in parentheses.
[c]l, larva; j, juvenile; m, metacercaria.

Results of discriminant analyses are presented as matrices showing the numbers and percentages of fish correctly and incorrectly classified by cross validation for the eight areas sampled and for the subsequent second set of analyses following the regrouping of collections.

Results

Eight-Category Discriminant Analysis

Results of nonparametric discriminant analysis using eight categories (Table 3) showed a high level of separation for fish from the Saguenay Fjord (100% correct classification; misclassification of only a single fish from all other collections to this area) and Hamilton Bank (96.7% correct classification; no other fish misclassified to this area). Fish from the four collections from the Gulf of St. Lawrence (Estuary, Sept-Îles, North Anticosti, and Esquiman Channel) could not be distinguished from one another, correct classification ranging from 100% for North Anticosti to 3.3% for Esquiman Channel, but with substantial misassignment between collections. No fish collected from the Gulf of St. Lawrence were misclassified to the three collections taken from the Atlantic Ocean proper (Cumberland Sound, Hawke Channel, and Hamilton Bank) or to the collection originating from the Saguenay Fjord. Although fish from Hawke Channel were accurately classified (100% correct), this collection could not be clearly separated from Cumberland Sound, with 70% of fish from this area being misclassified to it.

Four-Category Discriminant Analysis

Based on the above analyses, the eight collections were regrouped into four categories: (1) the Saguenay Fjord, (2) the Gulf of St. Lawrence (Estuary, Sept-Îles, North Anticosti, and Esquiman Channel), (3) Hamilton Bank, and (4) Cumberland Sound/Hawke Channel. Reanalysis of the data using four categories resulted in an overall percent correct classification of 99.6% (Table 4). All fish were correctly classified with the exception of a single fish from Hamilton Bank which was misassigned to the Saguenay Fjord.

The results demonstrate that parasites can be used to separate fish from the Gulf of St. Lawrence from those from adjacent waters of the Saguenay Fjord and the Atlantic Ocean off Labrador with an extremely high level of accuracy. Fish from the Gulf are characterized by high levels of infection with the nematode *Anisakis simplex* and metacercariae of the digenean *Otodistomum* sp. and relatively low levels of infection with the acanthocephalan *Corynosoma strumosum* and the nematode *Pseudoterranova decipiens* (see Table 2). Those from the Saguenay Fjord are characterized by high levels of *Corynosoma*, *Pseudoterranova*, and *Anisakis* and an absence of *Otodistomum* metacercariae. Fish from Hamilton Bank and Hawke Channel/Cumberland Sound are characterized by relatively low levels of infection by *Anisakis*, *Pseudoterranova*, and *Corynosoma* and the complete absence of *Otodistomum*.

Discussion

The ability to separate stocks on the basis of parasites reflects variation in the ecological conditions found in these regions and is a result of the nonuniform distributions and abundances of the parasites' final and, perhaps, first intermediate hosts. *Anisakis simplex*, also known as the "whaleworm," uses euphausiids as first intermediate hosts and fish as second intermediate and transport hosts (Oshima 1972). It develops to maturity in various cetaceans (see Margolis and Arai 1989). The high abundance of *Anisakis simplex* in collections in the Gulf of St. Lawrence is probably directly related to the large number of whales which frequent this region (see Mitchell 1973).

Pseudoterranova decipiens is believed to use various benthic invertebrates as first intermediate hosts and groundfish as second intermediate and transport hosts (McClelland 1990; McClelland et al. 1990). It develops to maturity in various pinnipeds, of which the grey seal (*Halichoerus grypus*) is the most important in Canadian Atlantic waters (see Bowen 1990).

The acanthocephalan *Corynosoma strumosum* uses amphipods as intermediate hosts, fish as transport hosts, and various seals (grey seal, harbour seal (*Phoca vitulina*), and bearded seal (*Erignathus barbatus*)) as final hosts (see Van Cleave 1953; Margolis and Arai 1989). Although seals are not particularly abundant in the Saguenay Fjord (M. Hammill, Maurice Lamontagne Institute, Mont-Joli, Que., personal communication), the high abundances of *Pseudoterranova* and *Corynosoma* in Greenland halibut from this region may be due to the presence of deep water close to their hauling-out sites, thus facilitating transmission. Also, small whales, such as the beluga (*Delphinapterus leucas*), may play an important role in the localized transmission of *Corynosoma*.

The complete life cycle of *Otodistomum* sp. has not been elucidated (Gibson and Bray 1977). The first intermediate host is probably a deepwater mollusc. Greenland halibut may act as

North Anticosti ($n = 30$)		Sept-Îles ($n = 30$)		Estuary ($n = 30$)		Saguenay ($n = 30$)	
P(%)	Intensity (range)	P(%)	Intensity (range)	P(%)	Intensity (range)	P(%)	Intensity (range)
100.0	6.8 ± 3.9 (1–16)	100.0	7.5 ± 6.4 (1–28)	100.0	16.5 ± 10.3 (3–38)	96.7	7.9 ± 5.8 (1–25)
0.0	—	3.3	1.0 ± 0.0 (1)	19.1	1.8 ± 1.0 (1–3)	93.3	9.4 ± 4.9 (1–18)
16.7	1.2 ± 0.4 (1–2)	16.7	2.2 ± 1.6 (1–5)	33.3	5.1 ± 2.4 (2–9)	63.3	1.5 ± 0.9 (1–4)
66.7	20.3 ± 18.6 (3–82)	43.3	44.0 ± 70.8 (1–217)	47.6	71.0 ± 73.8 (11–229)	0.0	—
90.0	4.5 ± 3.6 (1–18)	90.0	7.7 ± 11.8 (1–59)	81.0	6.6 ± 5.0 (1–17)	60.0	12.6 ± 15.9 (1–54)

TABLE 3. Results of nonparametric descriminant function analysis for the separation of Greenland halibut originating from localities off the Canadian northwest Atlantic using parasite data. Overall correct classification = 59.7% (138/231).

True category	Assigned category[a]							
	ANTI	CUMB	ESQ	ESTU	HAM	HAWK	SAG	SEPT
ANTI	30[b] 100.0%	0	0	0	0	0	0	0
CUMB	0	9 30.0%	0	0	0	21 70.0%	0	0
ESQ	29 96.7%	0	1 3.3%	0	0	0	0	0
ESTU	13 61.9%	0	0	6 28.6%	0	0	0	2 9.5%
HAM	0	0	0	0	29 96.7%	0	1 3.3%	0
HAWK	0	0	0	0	0	30 100.0%	0	0
SAG	0	0	0	0	0	0	30 100.0%	0
SEPT	25 83.3%	0	0	2 6.7%	0	0	0	3 10.0%

[a]ANTI = North Anticosti, CUMB = Cumberland Sound, ESQ = Esquiman Channel, ESTU = Estuary, HAM = Hamilton Bank, HAWK = Hawke Channel, SAG = Saguenay, SEPT = Sept-Îles.
[b]Indicates number of fish classified to category followed by percentage.

true second intermediate hosts, or possibly may acquire infections by feeding on infected crustaceans or on smaller fishes, thus acting as transport hosts. Adult *Otodistomum* are parasitic in the spiral valve or body cavity of elasmobranch fishes, while metacercariae are found in a variety of fishes (Gibson and Bray 1977). The restricted distribution of this parasite in Greenland halibut is probably related to the geographical distribution of the as yet unknown molluscan host. Scott and Bray (1989) noted the occurrence of *Otodistomum* in Atlantic halibut (*Hippoglossus hippoglossus*) from the Scotian Shelf (NAFO divisions 4W and 4Vs, with prevalences of 2 and 32%, respectively) and in one of nine Greenland halibut examined from 4Vs. Scott (1975) noted that the occurrence of metacercariae in witch flounder (*Glyptocephalus cynoglossus*) was considerably higher in fish from the Gulf of St. Lawrence than in those from the Scotian Shelf (35.0 versus 13.7%). *Otodistomum* metacercariae have not been reported in fishes collected farther north of the continental shelf than NAFO division 4Vs (Banquereau Bank).

Larval nematodes assigned to the tribe Contracaecinea as defined in Hartwich (1974) represent larval stages of the genera *Phocascaris* and *Contracaecum*, adults of which are parasites of various marine mammals, and in the case of the latter genus, also piscivorous birds. Morphological features necessary for genus and species identification are not developed in third stage larvae found in fish (Berland 1981; Valtonen et al. 1988). Material from Greenland halibut probably contains at least two species, *Phocascaris phocae* Host, 1932 and *Contracaecum osculatum* (Rudolphi, 1802), both common parasites of pinnipeds off Atlantic Canada. It is possible that additional species of *Contracaecum*, particularly those using piscivorous birds as definitive hosts, may also be contained in this taxon.

As noted by Lester (1990), analysis of geographical variation in the distribution and abundance of parasites is an excellent source of information on the movements of marine fishes. Such analyses can occasionally be used to discriminate between host populations, although unequivocal separation typically requires study of fish population genetics. The high level of separation achieved between Greenland halibut from the Gulf of St. Lawrence and those from adjacent areas of the Saguenay Fjord and the Atlantic Ocean proper (Hamilton Bank and Hawke Channel) indicates that there is little interchange of subadult or adult fish between these areas. Results of tagging experiments conducted

TABLE 4. Results of nonparametric discriminant function analysis for the separation of Greenland halibut originating from the Canadian northwest Atlantic following regroupment of the eight collections into four categories using parasite data. Overall correct classification = 99.6% (230/231).

True category	Assigned category[a]			
	Cumberland/Hawke	Gulf	Hamilton	Saguenay
Cumberland/Hawke	60[b] 100.0%	0	0	0
Gulf	0	111 100.0%	0	0
Hamilton	0	0	29 96.7%	1 3.3%
Saguenay	0	0	0	30 100.0%

[a]Cumberland/Hawke = Cumberland Sound and Hawke Channel; Gulf = Estuary, Sept-Îles, North Anticosti, and Esquiman Channel; Hamilton = Hamilton Bank; Saguenay = Saguenay Fjord.
[b]Indicates number of fish classified to category followed by percentage.

off the coast of Newfoundland and Labrador by Bowering (1984) support the hypothesis that there is little, if any, movement of large Greenland halibut from this region into the Gulf of St. Lawrence. The shallower water of the Strait of Belle Isle (maximum depth of 75 m) separating Esquiman Channel from nearby Hamilton Bank and Hawke Channel probably prevents older Greenland halibut from moving between these areas. The relationship of fish in the northeastern portion of the Gulf of St. Lawrence to those occurring off Newfoundland and Labrador was first investigated by Templeman (1970) who, based on differences in vertebral counts, hypothesized that spawning may occur in the Gulf of St. Lawrence. The genetic relationships of fish from these two areas were subsequently investigated by Fairbairn (1981), who examined the distribution of electrophoretically detectable protein variants. She concluded that Gulf fish were relatively isolated from the northwest Atlantic stock but that some gene flow occurred between the two areas, probably via the Strait of Belle Isle. Tremblay and Axelsen (1981) reached similar conclusions based on analyses of length/weight relationships and vertebral counts. These authors also concluded that in order to produce the observed differences in vertebral counts, any recruitment to the Gulf stock from the larger northwest Atlantic population would have to occur at the larval stage. These general conclusions have been supported and refined by the subsequent studies of Khan et al. (1982) using blood parasites, Bowering (1982, 1984) using tagging and catch statistics, and Misra and Bowering (1984) using meristic analyses. Our parasitological results are in agreement with the existing concept of the relationship of these two major stocks. They also clearly demonstrate that any movement of Greenland halibut from the larger population occurring off Labrador and Newfoundland into the Gulf of St. Lawrence must occur very early in the life history of this fish.

The relationship between the small stock of Greenland halibut occurring in the Saguenay Fjord and that part of the larger Gulf population found in the St. Lawrence Estuary has not been previously studied. The Saguenay Fjord is separated from the St. Lawrence Estuary by a shallow-water barrier (three ledges, the shallowest of which has a depth of only 18 m), the existence of a thermohalocline, and other hydrological features (see Drainville 1968; De Ladurantaye et al. 1984). These features appear to prevent the movement of subadult and adult Greenland halibut between these two areas, as is evidenced by the high level of separation achieved during this study. Electrophoretic analyses of allozyme patterns conducted in coordination with our parasitological studies indicate that there are no important genetic differences between the fish from the Saguenay Fjord and those from the St. Lawrence Estuary (J.-M. Sévigny, Maurice Lamontagne Institute, Mont-Joli, Que., personal communication). It is thus likely that recruitment to the Saguenay Fjord stock from the Estuary also occurs primarily through the movement of young stages.

Separation of Greenland halibut from Hamilton Bank from those from nearby Hawke Channel and from Cumberland Sound appears to be based mainly on the relatively high level of infection by Contracaecinea larvae in the latter two collections (see Table 2). These results indicate that fish from Hamilton Bank probably remain in a rather restricted geographical area during the period up to their spawning migration. Although the Hamilton Bank area is probably continually repopulated by juveniles carried southward by the Labrador current from each year's spawning in the Davis Strait, recovery of stocks in this area would be slow were it heavily fished because few older fish enter from adjacent areas. Riget and Boje (1989), in their summary of the biology of Greenland halibut in West Greenland waters, considered fish occurring in West Greenland fjords to be mainly stationary. Our parasitological findings indicate that this is probably also the case for fish occurring in the Saguenay Fjord and on Hamilton Bank.

As a result of similar prevalences and intensities of infection of the five parasite taxa used (see Table 2), fish from Cumberland Sound could not be distinguished from those from Hawke Channel. However, the great distance between these collections (about 1600 km) and evidence provided by other parasite species indicate that fish from these two areas are unlikely to mix prior to spawning. The latter, which were not used in analyses due to the conservative approach taken (see Materials and Methods), include the myxosporeans *Ortholinea divergens* sensu Auerbach, 1912, found in 60.0% of the fish from Hawke Channel but absent in those from Cumberland Sound, and *Myxoproteus reinhardti* (Wierzbicka, 1986), found in 96.7% of fish in Hawke Channel but only 10.0% of those from Cumberland Sound, and the digenean *Steringophorus fuciger* (Olsson, 1868), which infected 100% of fish from Cumberland Sound but only 13.3% of those from Hawke Channel. Although not supported by our statistical analyses, it seems likely, given the above differences,

that, like fish from Hamilton Bank, Greenland halibut from Cumberland Sound remain in a relatively limited geographical area prior to spawning.

Otodistomum sp. metacercaria, found in almost 60% of all Greenland halibut >39 cm sampled from the Gulf of St. Lawrence but absent in fish from all other collections, is a potentially useful tag for fish of Gulf origin. With appropriate sampling, it should be possible to determine whether or not these fish leave the Gulf to spawn with the larger stock in the Davis Strait and, if they do, to follow their migrations and determine the extent of mixing with other stocks.

In summary, analysis of the parasite fauna of Greenland halibut occurring off Atlantic Canada provides fisheries management with a powerful tool to study the discreteness of stocks and to follow host movements. Few, if any, large fish appear to move between the Gulf of St. Lawrence and adjacent areas of the Saguenay Fjord and off Labrador. The genetic flow observed between these two areas (Fairbairn 1981; J.-M. Sévigny, Maurice Lamontagne Institute, Mont-Joli, Que., personal communication) thus must result from the movement of early life history stages of this fish. It is also hypothesized that fish in some localities, such as Hawke Channel and Cumberland Sound, are much more stationary than previously believed. As there appears to be little recruitment of subadult fish into these areas, they may be severely affected by high exploitation rates which could cause temporary localized declines in abundance.

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Gammarus lawrencianus (Amphipoda) as Intermediate Host of *Echinorhynchus salmonis* (Acanthocephala) in an Estuarine Environment

Lena N. Measures[1] and Luci Bossé

Department of Fisheries and Oceans, Maurice Lamontagne Institute, P.O. Box 1000, Mont-Joli, QC G5H 3Z4, Canada

Measures, L.N., and L. Bossé. 1993. *Gammarus lawrencianus* (Amphipoda) as intermediate host of *Echinorhynchus salmonis* (Acanthocephala) in an estuarine environment. Can. J. Fish. Aquat. Sci. 50: 2182–2184.

Echinorhynchus salmonis (Acanthocephala) were found in the stomach of a rainbow smelt, *Osmerus mordax* (Salmoniformes), collected at the confluence of the Aux-Outardes River and St. Lawrence River in Quebec. One acanthocephalan occupied the haemocoel of a recently ingested amphipod (*Gammarus lawrencianus*), while two other specimens were free in the stomach. This is the first report of *G. lawrencianus*, an estuarine amphipod, as intermediate host of *E. salmonis*. This is also the first report of transmission of *E. salmonis* in an estuarine environment in North America.

L'acanthocéphale *Echinorhynchus salmonis* a été trouvé dans l'hémocèle de l'amphipode *Gammarus lawrencianus*. Ce dernier était présent dans l'estomac d'un éperlan arc-en-ciel, *Osmerus mordax* (Salmoniformes). L'éperlan fut capturé au confluent de la Rivière Aux-Outardes et du fleuve Saint-Laurent au Québec. Deux autres spécimens de cet acanthocéphale étaient libres dans l'estomac. Ceci constitue une première mention de *G. lawrencianus*, amphipode estuarien, comme hôte intermédiaire d'*E. salmonis*. C'est aussi une première mention d'une infestation par *E. salmonis* dans un milieu estuarien en Amérique du Nord.

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The acanthocephalan *Echinorhynchus salmonis* (Müller, 1784) is a parasite of freshwater and anadromous fishes of the Holarctic. A few strictly marine fish have also been reported as hosts of *E. salmonis*; however, it is primarily a freshwater–estuarine parasite (see Petrochenko 1956). In the Nearctic the freshwater amphipod *Pontoporeia hoyi* Smith, 1874 has been reported as an intermediate host of *E. salmonis* (Van Cleave 1920). Reports of amphipods naturally infected with acanthocephalans are rare. Herein, we report an amphipod, *Gammarus lawrencianus* Bousfield, 1956, infected with *E. salmonis* and two other free specimens of this parasite from the contents of the stomach of a rainbow smelt, *Osmerus mordax*, collected in an estuarine environment. Transmission of *E. salmonis* has not been reported previously in an estuarine environment in North America.

Methods

Rainbow smelt were collected at the confluence of the Aux-Outardes River and St. Lawrence River (49°4′19″N, 68°29′51″W) on 26 August 1992. The collection was made in a study of the environmental impact of damming the Aux-Outardes River. Rainbow smelt were captured by gill net in 2 m of water during low tide. Total length, weight, sex, and maturity of gonads were noted after which the stomach was removed and preserved in 10% formalin. Prey items were identified later in the laboratory using a dissecting microscope. Acanthocephalans found were stained with Schneider's acetocarmine and mounted on glass slides using standard parasitological techniques. Morphologic and morphometric studies of acanthocephalans were made using a Leitz Diaplan microscope equipped with a drawing tube coupled with a digitizer tablet and computer.

Voucher specimens of *E. salmonis* (CMNP1993-0041 and -0042) were deposited in the Canadian Museum of Nature, P.O. Box 3443, Station D, Ottawa, ON K1P 6P4, Canada, and in the parasitological collection at the Maurice Lamontagne Institute. The amphipod was deposited in the invertebrate collection at the Maurice Lamontagne Institute (72A-08802-8679).

Results and Discussion

One male *E. salmonis* was observed in the haemocoel of an amphipod found in the stomach of a rainbow smelt 215 mm long (Fig. 1). The amphipod measuring 8.75 mm from base of antenna to telson was identified as *G. lawrencianus* Bousfield, 1956. One male and one female *E. salmonis* were found free in the stomach of the same rainbow smelt. Gonads of the rainbow smelt were immature and sex was not determined. This rainbow smelt was an adult based on its total length (Scott and Scott 1988). The stomach was 50% full of prey items which consisted of 227 gammaridean amphipods and one each of a mysidacean, isopod, and fish. *Echinorhynchus salmonis* was not found in the stomachs of 25 other rainbow smelt examined. Sixty-two percent of the stomachs of the 26 rainbow smelt examined contained gammaridean amphipods.

Description of *E. salmonis*: Six spherical cement glands, not arranged linearly along midline. Ganglion near midlevel of proboscis receptacle. Trunk attenuated posteriorly. Longitudinal rows of hooks 15. Lemnisci 454–693 μm long. Proboscis

[1]Author to whom correspondence should be addressed.

receptacle 886–1000 µm long, 156–510 µm wide. Everted portion of proboscis (maximum everted 447 µm long) with hooks 41–53 µm long, bearing large roots. Male in haemocoel of infected amphipod: trunk 4.6 mm long, maximum width 488 µm; proboscis not completely everted (Fig. 1); anterior testis 594 × 263 µm, posterior testis 544 × 273 µm. Male free in stomach of rainbow smelt: trunk 5.1 mm long, maximum width 580 µm; anterior testis 241 × 433 µm, posterior testis 230 × 465 µm; proboscis partially everted. Female free in stomach of rainbow smelt: trunk 4.5 mm long, maximum width 504 µm; proboscis completed inverted; ovarian balls present.

Gammarus lawrencianus is a new intermediate host of *E. salmonis* and is also the first intermediate host from an estuarine environment in North America. *Echinorhynchus salmonis* has been reported from *Pontoporeia hoyi* Smith, 1874[2] from Lake Ontario (Van Cleave 1920) and *Pontoporeia affinis* Lindstrom, 1855[3] from Lake Huron (DeGiusi and Budd 1959), Cayuga Lake, N.Y. (Brownell 1970), and Lake Michigan (Amin 1978). *Pontoporeia affinis* Lindstrom, 1855 and *Pallasiola* (=*Pallasea*) *quadrispinosa* (G.O. Sars, 1867) have been identified as intermediate hosts in the Palearctic (Nybelin 1924, cited in Petrochenko 1956; Shulman 1954). Valtonen (1980) found *P. affinis* Lindstrom, 1855 infected with cystacanths of *E.* (=*Metechinorhynchus*) *salmonis* in Bothnian Bay, an oligohaline part of the Baltic Sea.

Gammarus lawrencianus is a benthic amphipod known to inhabit lotic waters but is mainly estuarine, tolerating salinities as low as 3–4‰ (Bousfield 1973). *Gammarus lawrencianus* occurs from Labrador to the Gulf of Maine inhabiting shallow saline pools in estuaries during winter and spring and lotic waters with low salinities during summer. The life cycle is annual and amphipods born in spring or summer overwinter in saline pools (Steele and Steele 1970). The infected rainbow smelt in this study was collected in the freshwater outflow of the Aux-Outardes River where it probably ingested the infected amphipod.

Anadromous rainbow smelt enter rivers in spring to spawn, their fry being found later in brackish water in May. During summer, rainbow smelt are found offshore in deeper and cooler water. The movement of rainbow smelt at sea is not well known but they enter estuaries in fall and late winter (Scott and Scott 1988).

The prevalence of *E. salmonis* was 51–67% in rainbow smelt collected in August 1979 and 1980 near Baie Comeau on the north shore of the St. Lawrence River (Fréchet et al. 1983), approximately 50 km downstream from the present study site. Rainbow smelt collected by Fréchet et al. (1983) near Forestville, approximately 50 km upstream from the present study site, had a prevalence of only 3% in June 1979 but this increased to 43–46% in August 1979 and 1980. Unfortunately, Fréchet et al. (1983) did not conduct a seasonal study of *E. salmonis* in rainbow smelt, nor did they report intensity and maturity of *E. salmonis* in rainbow smelt. All their specimens were found in the lumen of the intestine. As with the infections reported herein, it is likely that *E. salmonis* in rainbow smelt collected in August by Fréchet et al. (1983) were newly acquired infections. Our findings are relevant to fisheries research, as Fréchet et al. (1983) found *E. salmonis* useful as a biological tag of populations of rainbow smelt in the St. Lawrence River.

FIG. 1. *Gammarus lawrencianus* (Amphipoda) infected with *Echinorhynchus salmonis*. Arrowhead indicates partially everted proboscis. Scale bar = 1 mm.

In conclusion, we report a new amphipod intermediate host of *E. salmonis*. This study shows that transmission of *E. salmonis*. from infected *Gammarus lawrencianus* to rainbow smelt can occur in late summer in an estuarine environment. Further data are required to verify when the parasite is transmitted to rainbow smelt and amphipods in an estuarine environment and to determine whether rainbow smelt lose infections after spawning.

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[2]In a revision of the genus, Bousfield (1989) divided *Pontoporeia* into three genera, *Pontoporeia, Monoporeia,* and *Diporeia. Pontoporeia hoyi* Smith, 1874, which has been transferred to *Diporeia*, is distributed in freshwater glacial relict lakes of North America. All freshwater *P. affinis* reported infected with *E. salmonis* in North America are probably *Diporeia hoyi* (Smith, 1874).

[3]*Pontoporeia affinis* Lindstrom, 1855, which has been transferred to *Monoporeia*, is distributed in coastal marine, mainly brackish lakes and estuaries and marine glacial relict lakes in the Holarctic (Bousfield 1989).

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Lipid Dynamics during Reproduction in Two Livebearing Fishes, *Gambusia holbrooki* and *Poecilia latipinna*

Gary K. Meffe

University of Georgia's Savannah River Ecology Laboratory, Drawer E, Aiken, SC 29802, USA

and Franklin F. Snelson, Jr.

Department of Biology, University of Central Florida, Orlando, Fl 32816, USA

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In animals, strategies of energy allocation among growth, maintenance and reproduction can be significantly altered by lipid storage. Poeciliid (livebearing) fishes store energy in late summer and fall for overwintering and first reproduction in spring, but details of energy use in reproduction are lacking. We conducted a laboratory experiment on the eastern mosquitofish (*Gambusia holbrooki*) and the sailfin molly (*Poecilia latipinna*) to document changes in lipid content in both the ovary and soma during development of a brood. In females of both species, ovarian lipid content was highest early in embryogeny and then declined; adult somatic lipids increased (were replenished) during embryonic development in mosquitofish, but declined in mollies. Larger clutches sequestered a larger share of body lipids in both species, possibly indicating energetic limits to reproduction. Finally, growth rate was positively correlated with somatic lipid content in both species, indicating among-individual differences in metabolic efficiency or feeding efficiency rather than a trade-off between growth and energy storage.

Chez les animaux, les stratégies d'allocation de l'énergie entre la croissance, l'entretien et la reproduction peuvent être fortement modifiées par le stockage des lipides. Les Poeciliidae (poissons vivipares) stockent de l'énergie à la fin de l'été et en automne pour hiverner et se reproduire au printemps, mais nous manquons de détails sur l'utilisation de l'énergie dans la reproduction. Nous avons mené une expérience en laboratoire sur la gambusie de l'est (*Gambusia holbrooki*) et la molliénésie à voilure (*Poecilia latipinna*) pour étudier les variations de la teneur en lipides de l'ovaire et de l'ensemble du corps pendant le développement d'une couvée. Chez les femelles des deux espèces, la teneur en lipides des ovaires était au maximum au début de l'embryogénèse, puis baissait; chez les adultes, les lipides somatiques augmentaient (étaient renouvelés) pendant le développement embryonnaire chez la gambusie, mais baissaient chez la molliénésie. Les couvées de grande taille retenaient une portion plus grande des lipides somatiques chez les deux espèces, ce qui peut indiquer les limites énergétiques à la reproduction. Enfin, le taux de croissance était positivement corrélé à la teneur en lipides somatiques chez les deux espèces, ce qui indique l'existence de différences entre les individus dans l'efficience métabolique ou alimentaire plutôt qu'un compromis entre la croissance et le stockage de l'énergie.

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Energy assimilated by an animal may be allocated to one of four "compartments," growth, maintenance, reproduction, and storage, among which trade-offs may occur. For example, energy used in reproduction is unavailable for growth. If maintenance costs increase, storage may diminish. Allocation "decisions" to growth, maintenance, or reproduction need not be immediate, however, as energy may be stored when abundant and then used later when energy demands are high or resource availability low.

The role stored energy (typically in the form of lipids) plays in reproductive energetics is not clearly understood. Stored lipids may allow earlier or larger reproductive bouts when resources would be otherwise limiting (Hahn and Tinkle 1965; Reznick and Braun 1987; Meffe and Snelson 1993). In these cases, somatic lipids could accumulate when resources for adults are high but conditions for offspring are poor, such as late summer at higher latitudes. Lipids could then be mobilized in early spring when resources for adults may still be limiting but when resources for offspring would soon be abundant. However, details of lipid shunting from somatic to reproductive compartments, and of use within the latter, are generally lacking.

In livebearing fishes (Poeciliidae), several studies have addressed annual lipid cycles (Falke and Smith 1974; Bennett and Goodyear 1978; Reznick and Braun 1987; Meffe and Snelson 1993). These studies suggest that lipid storage occurs in late summer/autumn. Some stored energy is used to overwinter, and then lipids shift to ovaries in spring, at the onset of reproduction. However, nothing is known of lipid use within the ovary or of details of lipid shifts between the soma and ovary during an ovarian cycle. In this paper, we experimentally address lipid dynamics within a single ovarian cycle in the eastern mosquitofish (*Gambusia holbrooki*) and the sailfin molly (*Poecilia latipinna*).

Methods

Poeciliid Reproductive Biology

All but one of the nearly 200 members of the family Poeciliidae are livebearers. Females are internally fertilized, and embryogenesis occurs directly in the ovarian follicles. After a gestation period of 20–40 d (depending on species and temperature), precocial neonates are delivered.

Within the poeciliids, species range from those whose females produce large, yolky eggs with no subsequent maternal nutritive input (lecithotrophy) to those that produce eggs with virtually no yolk and then supply embryos with nutrition through a pseudoplacenta and other tissues (matrotrophy). In lecithotrophic species, reproductive energy demands on the female are largely pulsed, occurring over the short period of vitellogenesis and egg maturation; in matrotrophs, instantaneous demands are lower and spread throughout gestation (Constantz 1989).

Both the eastern mosquitofish (Reznick 1981) and the sailfin molly (Amoroso 1960) are lecithotrophic species. Consequently, high reproductive energy demands occur during vitellogenesis, a few days after parturition (Snelson et al. 1986). After fertilization, the embryos gestate for some 25–30 d.

Experimental Design

Because measuring lipid content necessitates destruction of the individual, determination of lipid dynamics requires study of a population (i.e., lipid levels cannot be measured repeatedly in an individual). We therefore isolated individual pregnant females of both species until parturition of a brood, which was designated as "day 0," or the starting point of the next brood cycle. We then continued holding them for specified periods (0, 3, 7, 14, 21, or 28 d, chosen to cover the expected gestation period) into the next brood, at which point they were sacrificed for lipid analysis. In this way, we could determine somatic and ovarian lipid levels at specific points in the ovarian cycle, using different individuals at each point.

We used 96 females per species, each placed in a 3.8-L plastic milk container, whose top had been removed and sides had been cut out and covered with nylon window screen. Numbered containers were placed in one of eight 225-L glass aquaria (four aquaria per species, 24 containers per aquarium), supplied with filtration and submersible heaters. This design allowed individual feeding of fish held in a common water bath. With six sampling periods (randomly assigned to females within a tank), the design produced 16 females at each period.

Treatment of Fish

Sailfin mollies were collected from a roadside ditch at Titusville, Florida, on 25 February 1989. They were held at 18°C and 5% salinity because mollies typically fare poorly in freshwater. Mosquitofish were collected from Risher Pond (Meffe 1990, 1991) on the U.S. Department of Energy's Savannah River Site, near Aiken, South Carolina, on 28 February 1989. They were also held at 18°C, but at zero salinity. During acclimation, from collection to 8 March, they were fed flake food and frozen brine shrimp ad libitum; they ate this laboratory food readily.

On 8 March 1989, heaters were added to the tanks and temperatures were increased over 1 wk to 25–26°C to simulate spring conditions and initiate reproduction. Courtship and copulation quickly ensued in both species, and females began to show signs of pregnancy. Ninety-six females of each species were stocked into their individual containers on 21 March. Experimental tanks were held at 25–26°C; temperatures were checked twice daily and adjusted as needed. Mollies were kept at 5% salinity and mosquitofish at 0%. For the remainder of the study, all fish were checked twice daily for condition and parturition and fed flake food ad libitum for three meals and frozen brine shrimp for the fourth.

Upon parturition, neonates were removed and the parturition date used as the female's "day 0". She was weighed to the nearest 0.01 g in a tared beaker of water on a digital electronic balance and measured (standard length, SL) to the nearest 0.1 mm with dial calipers. She was then returned to her container and maintained as above until the preassigned sampling day, at which time she was again weighed and measured, killed in 20% formalin, and stored in 5% buffered formalin. There was no need to provide access to males during the experiment, as both species store sperm through several brood cycles (Constantz 1989).

We measured female growth rate as mean change in weight between day 0 and sampling days 7, 14, 21, and 28. We did not use growth estimates for fish sampled on day 0, as they had no opportunity to grow, or day 3, because the small amount of growth would impart too large a measurement error component. We also measured growth in length, but results were qualitatively the same as weight and will not be presented.

The design worked well for sailfin mollies, but all fish in two of four mosquitofish tanks died within 3 wk. Post mortem investigations were inconclusive. We believed we could not trust the data from the other two tanks, so we restarted the mosquitofish experiment on 27 April with a new set of fish, collected from Risher Pond 6 d earlier. Within 2 wk, fish in two of those tanks also began to die. We then discovered that mortality was due to a buildup of ammonia and nitrites in those tanks, and we immediately placed ammonia-removing filters in the remaining two tanks. All remaining mosquitofish were unaffected by this problem and were healthy throughout the experiment. Thus, the sample size for this species was 48 rather than 96.

Gravimetric Lipid Analysis

Within 6 mo of preservation, fish were dissected to remove the ovary and clean the gut of food in preparation for gravimetric lipid analysis. Pilot studies indicated that preservation for up to 1 yr has no effect on lipid quantification (G.K. Meffe, unpublished data). Eggs/embryos were separated, counted, and staged according to Meffe (1985). Stages are as follows: 1, immature ova; 2, mature ova/fertilized egg; 3, primitive streak; 4, early eyed; 5, middle eyed; 6, late eyed. The soma (diced) and ovaries were put into separate, dried, and tared cellulose extraction thimbles (Fisher Scientific), placed in a drying oven for 2 d at 55°C, transferred to a desiccator at 0–0.5% humidity for 2 d, and placed in a desiccated glove box at 0–0.5% humidity for another day. They were then weighed to the nearest 0.01 mg on a Mettler balance inside the glove box.

Samples were extracted in anhydrous (ethyl) ether at 80°C, using a Soxtec® HT2 extractor, model 1045. Lipid extraction involved boiling sample thimbles in the solvent for 1 h followed by rinsing in distilled solvent for 1 h. After extraction, thimbles were again dried and weighed as above. The lipid content was calculated as the difference between fat and lean weights. Further details of this technique are provided in Meffe and Snelson (1993).

All statistical and graphical analyses were conducted with Systat® and Sygraph® software (Wilkinson 1990). We initially

conducted all analyses with "time since parturition" and "stage of development" as independent variables, but since both procedures led to the same conclusions, we present only analysis of stage of development. Data were first checked for normality wherever appropriate and did not require transformation.

Results

All 96 mollies lived for their assigned time in the experiment. Of the 48 mosquitofish, one was dropped because of an obvious weighing or recording error. All other fish appeared normal and produced healthy initial broods.

Choice of longest sampling interval after first parturition was the result of an educated guess to compromise between taking fish too early (before gestation was near completion) and letting them develop too far, thereby risking a second parturition. The 28-d period proved appropriate for mosquitofish, as a large number of fish were represented at each point. However, the 28-d period was too short for mollies, as only one molly clutch reached the final stage of development in that time.

Variation in regressions of percent lipid on other parameters was great. Although immediate sources of variation (recent history of the fish; food type and amount; temperature) were controlled, there are other sources that were uncontrollable with this design (longer term history of the fish, including previous reproduction; age; genetic constitution). Further, there simply may be high interindividual variation in a trait such as lipid content. In spite of this variaton, there were patterns in the lipid data.

Percent lipid of the ovary was significantly negatively correlated with stage of development in eastern mosquitofish, but only marginally so in sailfin mollies (Fig. 1), indicating that lipid content in the ovaries was highest at the beginning of a brood and became depleted during gestation. Percent lipid of the soma was significantly positively correlated with stage of development in mosquitofish, but negatively so in mollies (Fig.1). This indicated that during gestation, somatic lipid was replaced in mosquitofish but declined in mollies.

For analyses involving clutch size, we eliminated individuals with zero clutches (individuals that had not completely yolked ova). Because development of the next clutch had not yet started, we would not know how the somatic lipid present at that time might later be distributed among subsequent ova.

Percent lipid of the ovary was negatively correlated with clutch size in mosquitofish but not in mollies (Fig. 2). Neither of the somatic lipid/clutch size correlations was significant. Thus, larger clutches of mosquitofish had lower percentages of lipid, suggesting that lipid was limiting; this relationship was absent in mollies. There was no consistent relationship between somatic lipid and clutch size in either species.

The percentage of total available body lipid contained in the ovary ("ovarian lipid allotment") indicated the extent to which reproduction sequestered lipid reserves. In both species, there was a significant, positive relationship between ovarian lipid allotment and clutch size (Fig. 3). That is, larger clutches contained a greater share of total available body lipid. The relationship appeared stronger for mosquitofish (higher r) than for mollies.

The correlation between ovarian lipid allotment and total lipids available in the body was significantly negative for both species (Fig. 4). This means that when lipids were abundant (e.g., 20% total available lipids), the ovary sequestered a larger proportion of total lipids available for reproduction. These negative

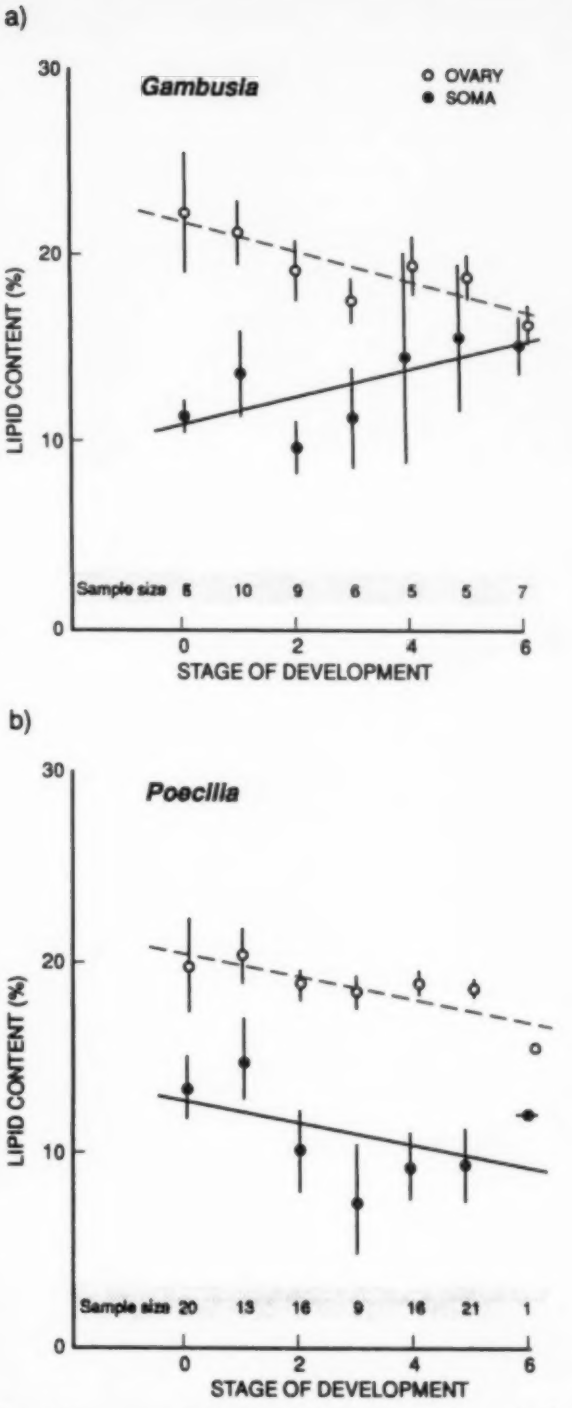

FIG. 1. Mean percent lipid content (±2 SE) in soma and ovaries for (a) *G. holbrooki* and (b) *P. latipinna*. Sample sizes are shown at the bottom of each figure. Spearman's (nonparametric) correlation coefficients of the individual data points (means shown for clarity) were used because of the categorical nature of the abscissa and are (a) soma: $r_s = 0.331$, $P < 0.05$; ovary: $r_s = -0.558$, $P < 0.01$, n = 47 and (b) soma: $r_s = -0.424$, $P < 0.001$; ovary: $r_s = -0.233$, $P < 0.05$, $n = 96$.

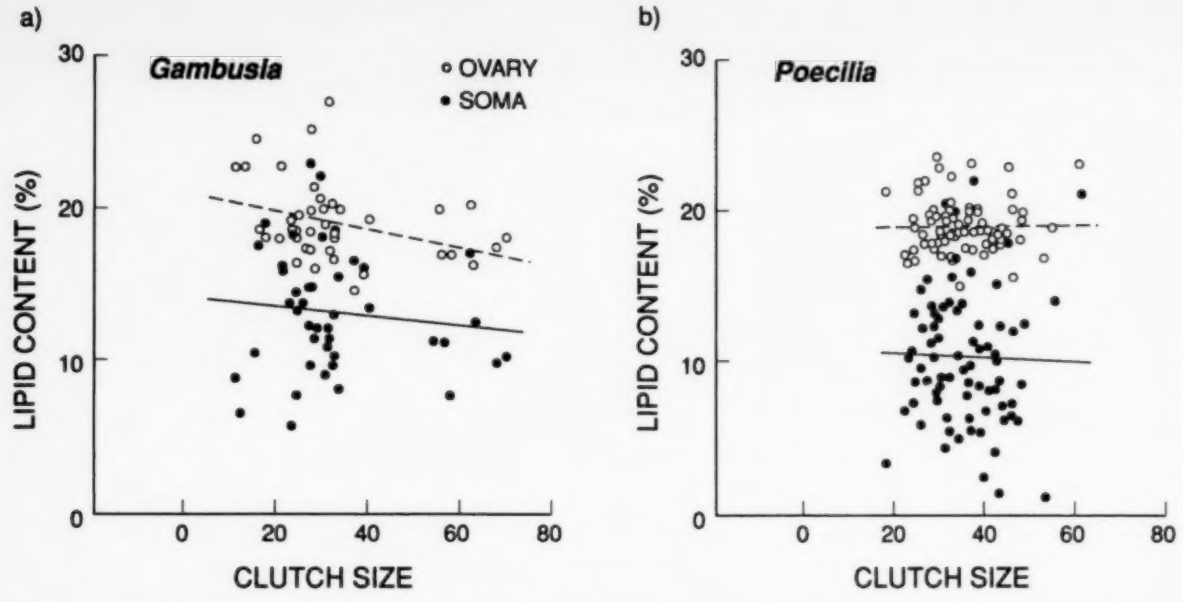

FIG. 2. Percent ovarian and somatic lipid content as a function of clutch size in (a) *G. holbrooki* and (b) *P. latipinna*. Pearson's correlation coefficients are (a) soma: $r = -0.11$, n.s., ovary: $r = -0.32$, $P < 0.05$ and (b) soma: $r = -0.02$, n.s., ovary: $r = 0.04$, n.s.

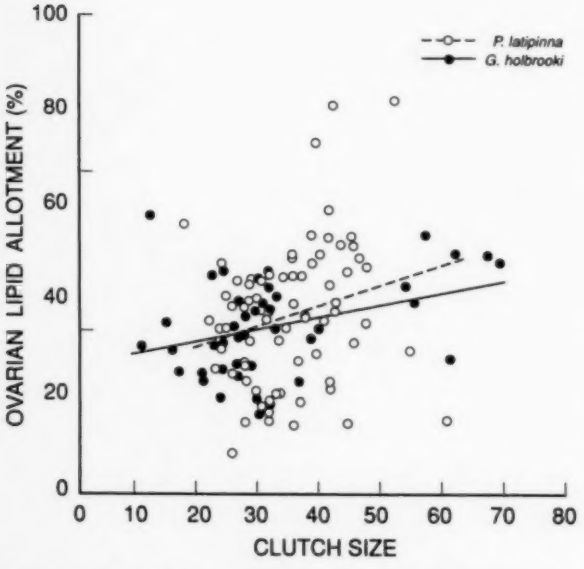

FIG. 3. Ovarian lipid allotment (percentage of total body lipid present in the ovary) as a function of clutch size for *G. holbrooki* ($r = 0.34$, $P < 0.05$) and *P. latipinna* ($r = 0.24$, $P < 0.05$).

correlations are especially meaningful because ovarian and somatic lipid contents are either uncorrelated (mosquitofish: $r = -0.079$, $P > 0.05$, $n = 47$) or significantly positively correlated (mollies: $r = 0.216$, $P < 0.05$, $n = 96$). Despite that, negative correlations exist between the total amount of lipids available and the amount in the ovaries. Both species thus may face a reproductive lipid limitation when overall lipid content of the body is low.

Mean embryo size was measured as ovarian dry weight (pre-extraction) divided by clutch size. Although this included a small amount of ovarian tissue, this is a tiny proportion of the overall weight and relatively constant across clutches. Mean embryo size in both species increased slightly or remained constant through gestation after reaching the mature ovum size at stage 2, but molly embryos were heavier than mosquitofish embryos (Fig. 5).

A final relationship of interest is that between adult lipid levels and adult growth rate during the experiment because energy that went into growth obviously could not be used for storage. In both species, somatic lipid level was significantly positively correlated with growth rate (Fig. 6); this relationship was stronger (steeper, and higher R^2) in mosquitofish. Correlations of somatic lipids and growth rate were unaffected by gestation time, measured as developmental stage, because partial correlations of somatic lipids and growth (with developmental stage partialed out) were essentially unchanged from the standard correlations. Fish with more somatic lipids grew faster than those with lower lipid levels.

The relationship between ovarian lipid levels and growth in mosquitofish was not significant (Fig. 6a) whereas ovarian lipid content in mollies showed a slight but significant decline with somatic growth (Fig. 6b).

Discussion

Previous research with mosquitofish from this population (Risher Pond) and another at the Savannah River Site (Pond C) indicated that stored lipids shift fom soma to ovary at the beginning of the reproductive season in March or April (Meffe and Snelson 1993). Other data in that study also indicated a

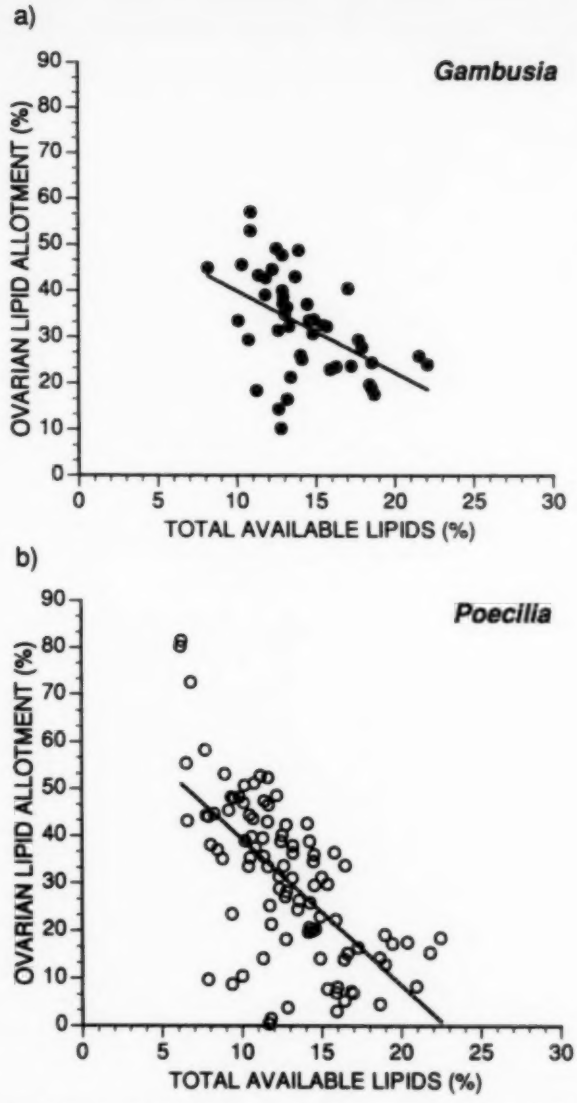

FIG. 4. Ovarian lipid allotment as a function of total available lipids for (a) *G. holbrooki* ($r = -0.481$, $P < 0.01$) and (b) *P. latipinna* ($r = -0.650$, $P < 0.01$).

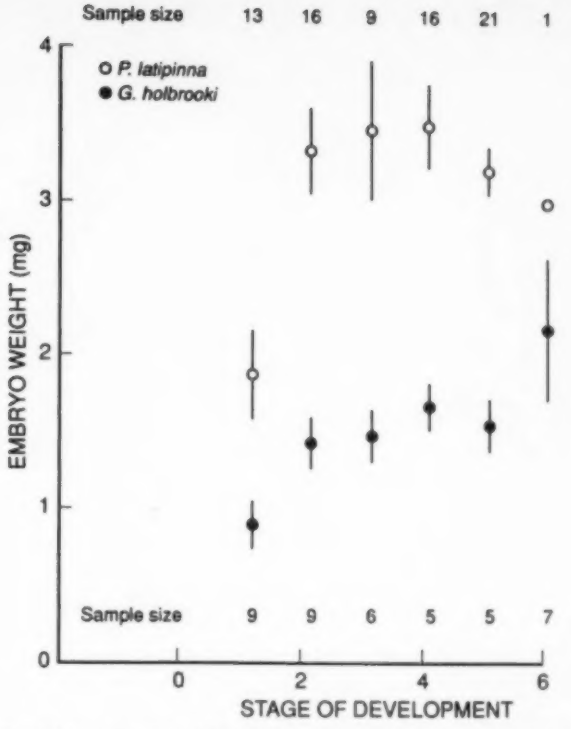

FIG. 5. Mean embryo weight (±2 SE) per each stage of development for *G. holbrooki* (sample size at bottom) and *P. latipinna* (sample size at top).

trade-off between somatic lipid storage and use of lipids in reproduction. The dynamics of lipid storage and use within a single ovarian cycle were also of interest, but were previously unknown. The present study addressed this gap by examining lipid patterns in the ovary relative to a single gestation period by delineating the patterns of percent lipid in the ovary and soma relative to gestation time, clutch size, and growth.

Ovarian percent lipid decreased during gestation in both species, indicating that lipid was metabolized by embryos during development. Because yolk is the main energy source during embryogenesis and has a high lipid content, this result is not surprising.

In adult mosquitofish, somatic lipid content increased during gestation, apparently being replaced after the initial shunting from storage lipids to eggs. However, in sailfin mollies, somatic lipid content significantly decreased during gestation. We propose two possible explanations for this. First, the mollies may have continued to provide nutrition to the developing embryos, at a cost of stored somatic lipids. Although both species are typically referred to as lecithotrophic (Amoroso 1960; Reznick 1981; Meffe 1987), it may be that mollies were to some degree matrotrophic. Trexler (1985) provided evidence that matrotrophy may occur facultatively in this species. The other possibility is that they simply did not receive enough food during the experiment, an unlikely event, as fish were fed large amounts, routinely were observed feeding, and food was typically left over after a feeding. However, the captive conditions may have behaviorally prevented fish from eating as much as they otherwise could, causing mobilization of stored lipids for maintenance energy.

The possibility of facultative matrotrophy may be further addressed by looking at mean embryo size through gestation. A lecithotrophic embryo typically loses 30–40% of its weight from the mature ovum (stage 2) to parturition (Scrimshaw 1945; Reznick 1981). If matrotrophy is involved, then less weight loss or even a weight gain is expected (Constantz 1980). In fact, molly embryos did have a constant mean weight through development from stage 2 (mature ovum) onward (Fig. 5; $r_s = -0.099$, not significant), indicating maternal energetic input during gestation. This supports Trexler's (1985) claims of facultative viviparity in sailfin mollies and may explain the

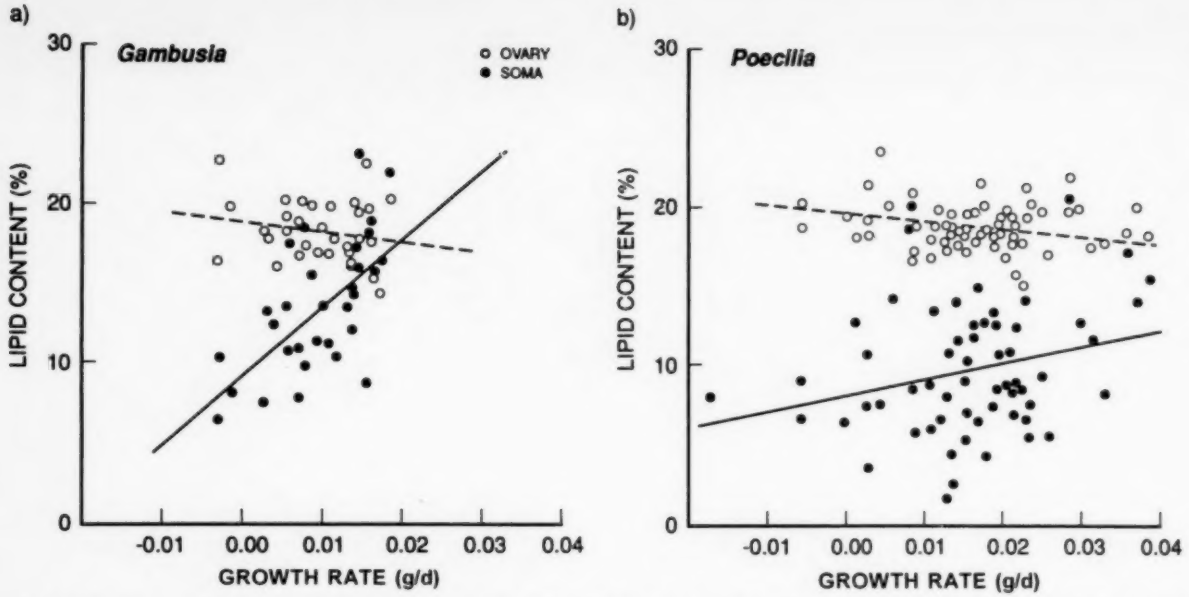

FIG. 6. Somatic and ovarian percent lipid content as a function of mean daily gain in weight for (a) *G. holbrooki* and (b) *P. latipinna*. Pearson's correlation coefficients are (a) soma: $r = 0.62$, $P < 0.01$; ovary: $r = -0.17$, n.s. and (b) soma: $r = 0.26$, $P < 0.05$; ovary: $r = -0.29$, $P < 0.05$.

continued loss of somatic lipid during gestation in this experiment (Fig. 1). However, mosquitofish embryos had a significant mean weight increase from stage 2 through 6 (Fig. 5a; $r_s = 0.638$, $P < 0.01$), indicating continued maternal energy commitments beyond vitellogenesis. Yet, mosquitofish adults also replaced somatic lipid during gestation (Reznick 1981; Meffe 1987). These results seem enigmatic.

It may be that the laboratory situation altered normal field patterns in both species. In a previous study, Meffe (1987) demonstrated a 33% weight loss in embryos from stage 3 (full-sized egg, primitive streak) to stage 6 (pre-parturition) in a wild mosquitofish population. However, in two controlled laboratory experiments, the decreases were only 14.3 and 25.1%. Furthermore, the absolute size of stage 3 embryos was 19.5% larger and of stage six embryos was 43% larger in the laboratory than in the field individuals. These results indicate that laboratory-reared fish produced larger initial eggs and may have added nutriment during development whereas the perhaps more energy-limited wild individuals produced smaller eggs and were not able to provide further energy during gestation. Alternatively, because the field and laboratory fish in Meffe (1987) came from several localities, those results may simply reflect population differences in these characters. Regardless, these results in aggregate indicate a great deal of reproductive variation in mosquitofish, either as individual plasticity or interpopulation differences.

Larger clutches had a lower percent ovarian lipid content than smaller clutches in mosquitofish, but not in mollies, indicating that mosquitofish energy budgets may have been more limited than molly budgets. Larger mosquitofish clutches had less lipid per embryo than did smaller clutches; in mollies, individual embryos in larger clutches did not experience this limitation.

The percentage of total available lipids garnered by reproduction increased with clutch size in both species (Fig. 3). This indicates a real lipid "cost" of reproduction and a further possible energy limitation. A greater percentage of overall body lipids was shunted to the ovary when clutches were large. Likewise, a greater proportion of overall lipid was found in the ovaries when total lipid content was low (Fig. 4), further indicating energy limitations on reproduction.

A priori predictions of relationships of somatic or ovarian lipid content with growth rate could range widely. A negative correlation could exist between growth and somatic or ovarian lipid content, indicating strong trade-offs between growth on one hand and storage or reproduction on the other. Alternatively, growth, reproduction, and storage may all be similarly influenced by overall metabolic efficiency, with more efficient individuals able to concurrently store energy, grow, and reproduce, which would produce positive correlations between growth and lipid content (Houle 1991).

In both species, growth was positively correlated with somatic lipid content (Fig. 6), supporting the metabolic efficiency argument. Some individuals were able to grow well while also storing body lipids whereas others were poor at both processes. Both species had a negative correlation between ovarian lipid and growth rate, significantly so in mollies, indicating a trade-off between growth and reproduction.

The positive correlation of growth rate and energy storage begs the question of why selection does not eliminate the slow-growing, low-lipid-storing genotypes. Those that grow fast and also store more lipids would seem to be at a strong selective advantage. However, these parameters were measured in only one environment. The "poor" genotypes in this study could be superior in a different setting, such as a low-food evironment.

Our study provides only an initial understanding of the role of lipids in the reproduction of poeciliid fishes. It is apparent that, at least in mosquitofish, some lipids move from storage to the ovary at vitellogenesis and are later replenished in the soma. It is not apparent what proportion of vitellogenic lipids are taken

out of storage, nor whether further lipid shunting occurs during embryogenesis. It is also not evident why there is so much among-individual variation in lipid levels under the controlled experimental conditions used. Further experimentation is needed to clarify these relationships. In particular, tight control of the genetic and evironmental backgrounds of experimental individuals would address the question of variation in lipid content. Experimental work with radioactively labeled lipids to track mass movement would verify lipid shunting among compartments and allow direct quantification of lipid dynamics.

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Patterns in the Size Structure of Littoral Zone Macroinvertebrate Communities[1]

J.B. Rasmussen

Department of Biology, McGill University, 1205 Dr. Penfield Avenue, Montreal, QC H3A 1B1, Canada

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A wide range of size spectra was encountered in 11 lakes of the Quebec Eastern Townships; most were bimodal with maximum biomass represented between 1 and 4 mg (wet weight) and between 64 and 256 mg and minimum biomass between 8 and 32 mg. PCA was used to show that the two size modes represented independent size fractions and that they were related to different environmental factors. The biomass of the small size fraction increased strongly with submerged macrophyte biomass and with lake trophic status. The biomass of the large size fraction was independent of the small size fraction and increased with wave exposure at the site. As the macrophyte biomass increased, more of the invertebrates were found upon the plants, and the small size fraction became more dominant. This is probably a result of the animals upon the vegetation being more exposed to fish predators than those hidden within the substrate. Linear regression models are developed to predict the biomass of both size fractions as well as the slope and intercept parameters of the normalized size distribution (NSD). The size-fraction approach (using PCA) complements the simpler NSD approach; it detects functionally independent size fractions in the community that NSD parameters cannot reveal.

Nous avons observé une vaste gamme de spectres de taille dans 11 lacs des Cantons de l'Est, au Québec; la plupart présentaient un double mode, avec un maximum de biomasse entre 1 et 4 mg (poids humide) et entre 64 et 256 mg et un minimum de biomasse entre 8 et 32 mg. La méthode PCA a servi à montrer que les deux modes de taille représentaient des fractions de taille indépendantes, et qu'ils étaient liés à des facteurs environnementaux différents. La biomasse de la fraction de petite taille augmentait fortement en même temps que la biomasse des macrophytes immergés et en fonction de l'état trophique du lac. La biomasse de la fraction de grande taille était indépendante de la fraction de petite taille, et augmentait avec l'hydrodynamisme du site. À mesure qu'augmentait la biomasse de macrophytes, la plupart des invertébrés se trouvaient sur les plantes, et la fraction de petite taille dominait davantage. De phénomène s'explique probablement par le fait que les animaux établis sur la végétation sont plus exposés à la prédation par les poissons que ceux qui sont cachés dans le substrat. Nous élaborons des modèles de régression linéaire pour prédire la biomasse des deux fractions de taille, ainsi que la pente et les paramètres de la distribution normalisée par taille (NSD). L'approche par fraction de taille (à l'aide de la méthode PCA) complète l'approche NSD, qui est plus simple; elle détecte les fractions de taille fonctionnellement indépendantes dans la communauté, ce que ne peuvent pas révéler les paramètres de la NSD.

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Biomass–size spectra are being used increasingly to describe, compare, and make predictions about aquatic communities. Being a nontaxonomic mode of analysis, it permits community analysis at a general level and thus provides an alternative and complementary perspective to taxonomic analysis. The size-based approach to pelagic ecology owes its appeal to a few simplifying features such as automated measurement (e.g., Coulter counting (Sheldon et al. 1972) and hydroacoustics (Sprules et al. 1991), its empirical simplicity (i.e., the "Sheldon spectrum" (Sheldon et al. 1972) characterized by uniform biomass across logarithmic size categories), and its theoretical appeal that is facilitated by the consistent predator–prey size ratios of pelagic food webs (Kerr 1974; Borgmann 1987). Strayer (1991) has correctly pointed out that none of these appealing features apply to benthic communities. Automated measurement is not and likely never will be possible. The size spectra that have been studied are usually not uniform (Schwinghammer 1981; Warwick 1984; Strayer 1986; Hanson et al. 1989; Hanson 1990; Morin and Nadon 1991), and the food webs appear to lack the consistent predator–prey size ratios characteristic of those in the pelagic zone. Indeed most large benthic organisms are microphagous (filter-feeders and deposit-feeders), and only a rather small percentage are macrophagous predators. In spite of this, the popularity of size-based approaches as used in pelagic systems has attracted benthic ecologists; however, the field is still in its early stages of development, and most papers have little data and much speculation.

One of the most appealing features of plankton size spectra, especially those from the sea (Sheldon et al. 1972, 1977), is their uniformity, which makes parameter estimation very simple. When biomass distribution across the logarithmic size categories is not constant, but follows a size trend, the slope parameter of the normalized size distribution (NSD) can be used to assess the

[1]A contribution from the Limnology Research Centre, McGill University.

degree of departure from the uniform biomass or "steady-state" distribution (Sprules and Munawar 1986). A slope of −1 indicates a tendency toward uniform biomass, slopes shallower than −1 indicate a trend of biomass increasing with size, and slopes steeper that −1 indicate a decreasing trend. Further, these authors showed that NSDs from open-water plankton communities had wide variation in their slopes and that this variation could be explained by ecosystem productivity, lake size, and the degree of perturbation experienced by a community. While the NSDs of some littoral macroinvertebrate communities have been analyzed (Hanson 1990), there are insufficient data on littoral communities to permit a comparative analysis of their size spectrum parameters. In this paper, I present analyses of the within- and between-lake variability of size-spectrum parameters for littoral macrozoobenthic communities and show that the community size structure is strongly linked to littoral habitat features.

Hanson (1990) compared the littoral invertebrate size structure at a site dominated by rooted plants (mostly *Potamogeton*) with that of beds dominated by *Chara* and argued that the nature of the macrophyte community was an important determinant of the littoral invertebrate size structure. He showed that the slope of the NSD was significantly different for communities in *Chara* beds (−0.89, i.e., biomass increasing with size) and those in *Potamogeton* beds (−1.11, i.e., biomass decreasing with size) within the same lake. This is as wide as the range reported for a variety of pelagic systems by Sprules and Munawar (1986). Hanson (1990) hypothesized that littoral zoobenthic size spectra would in general be as variable within lakes as between, especially in large lakes with variable macrophyte communities. Thus, we should expect to find very high variability in littoral invertebrate size structure within Lake Memphremagog, which exhibits extreme variability in weed-bed and substrate conditions, due to a high range of slopes and exposures, and the presence of a gradient of water clarity and trophic status along the lake (Duarte and Kalff 1986, 1990; Rasmussen 1988a, 1988b). Rasmussen (1988a) analyzed the littoral zoobenthic communities of Lake Memphremagog and a number of smaller lakes in the Eastern Townships of Quebec and presented models predicting community biomass from physical, chemical, and trophic factors. In this paper, I quantify the biomass size spectra from these communities and demonstrate patterns (both within- and between-lake) in the variation. Potential correlates of size structure that were analyzed at all study sites are the seasonal mean biomass of aquatic macrophytes, the type of macrophyte community present, degree of exposure to wind and waves, slope of the bottom, Secchi transparency, water colour, chlorophyll *a*, calcium content, and the redox potential and water content (coarseness) of the sediment.

Many of the normalized size spectra that have been used to estimate such slopes have distinct peaks and troughs rather than linear trends in biomass across size categories (Sprules et al. 1983; Warwick 1984; Sprules and Munawar 1986; Hanson et al. 1989; Hanson 1990), and thus the linear models applied to estimate the parameters sometimes fit rather poorly. Residuals around the fitted lines are highly autocorrelated and the poor statistical fit can lead to nonrobust parameter estimates when peaks and troughs are severe. In addition to these statistical problems, the fitting of NSD slopes to size spectra does not allow the detection of functionally independent size fractions, and in situations where peaks and troughs are strongly developed, the possibility that the peaks correspond to independent size fractions should not be overlooked. It has always been assumed that the size spectrum represents a functionally integrated trophic web (Kerr 1974; Borgmann 1982) where predator–prey size ratios are regular and both the overall biomass and the biomass in each size interval are determined solely by the productivity of the system. As I argued above, there seems little reason to believe that benthic systems are that simple. Indeed, Rasmussen and Kalff (1987) and Rasmussen (1988a, 1988b) found that lake trophic factors, while making an important contribution to the prediction of zoobenthic biomass, are generally less important than lake morphometry. Thus, I would expect that trophic coupling in the zoobenthos will be much weaker than that in the pelagic community and that the size spectrum will be less integrated, both functionally and statistically.

While I have argued that there are some statistical and conceptual difficulties with the NSD approach, I have retained it in my analyses because of its extreme simplicity. To complement the NSD approach, I have used principal components analysis (PCA) for post hoc indentification of independent size fractions and aimed at circumventing both the statistical problems that arise when NSD parameters are fitted to irregular size spectra (i.e., containing peaks and troughs) and the inability of the NSD approach to detect functionally distinct size fractions. This multivariate approach capitalizes on consistencies across lakes in the positioning of peaks and troughs within invertebrate size spectra and on the presence of consistent environmental determinants for each of the orthogonal size fractions. Regression models predicting NSD parameters from environmental factors will then be presented alongside models predicting the total biomass of the post hoc orthogonal size fractions.

Methods

Sample sites were chosen with a view to maximizing variability in morphometry, weed-bed characteristics, and water chemistry. Lakes that were small and had rather uniform littoral habitats were sampled at only one site whereas more sites were sampled in lakes that offered a greater diversity of littoral conditions. Half of the sites sampled ($N = 29$) were situated within the oligomesotrophic Lake Memphremagog (Quebec–Vermont), which varies greatly in littoral morphometry and wind exposure among its many bays and along its 45-km length. An additional 28 sites from 10 smaller lakes in the Quebec Eastern Townships were also sampled. These lakes (Rasmussen 1988a, 1988b) ranged from oligotrophic to strongly eutrophic and also presented a broad range of littoral slopes and exposures. At each site, a transect was echo-sounded (Honda-Sitex, model HE 356) perpendicularly from shore to the outer edge of the littoral, and sampling was done at approximately the midrange of the transect where plant growth was maximum.

Quantifying the Size Structure of the Littoral Zoobenthos

The littoral zoobenthic community includes both animals that live in association with the bottom and those associated with vegetation. Because the partitioning of the fauna between these two substrates is highly variable spatially and temporally, I treated the littoral zoobenthos as a single, vertically integrated community and quantified its abundance as biomass wet weight per square metre.

Biomass of epiphytic invertebrates (milligrams wet weight per gram wet weight of macrophytes) was estimated using the hand-held box sampler described by Rasmussen (1988a). A SCUBA diver enclosed a sample of undisturbed vegetation and trimmed

off all plants protruding from the sampler. As the sampler was removed from the water, the water inside was allowed to drain through a 0.2-mm screen on its side, and the contents of the sampler were placed into a plastic bag. In the laboratory, the vegetation was rinsed free of animals, marl, and most periphyton, identified to species, spun for 1 min in a salad spin-dryer, and weighed (wet weight in grams). The material washed from the plants, including the animals, was preserved in 5% formalin. On each sampling date when vegetation was present, two such samples were taken in the middle of the canopy, two within 50 cm of the substrate, and two within 1 m of the surface, if the canopy grew that high.

The total biomass of the submerged macrophytes at each site was estimated by taking five replicate 0.1-m^2 quadrat samples that were randomized by tossing the quadrat from a boat within the area of maximum canopy height. Samples were rinsed free of debris, indentified to species, spun to remove excess water, and weighed. The SCUBA diver also collected five 40-cm^2 cores of the sediment at each site, and these were washed on a 0.2-mm screen. The sieve residue, containing the animals, was preserved in 5% formalin. Animals were picked from the samples under a dissecting microscope (6×) and their lengths measured with an image analysis system (CORECO Inc., St. Laurent, Quebec, equipped with an Oculus 200 digitizer board). Fresh weights were estimated from total body lengths from a series of length–weight regressions constructed from the animals collected during this study. The estimates of weight from length were corrected for back-transformation bias arising from the use of log-linear regressions as suggested by Bird and Prairie (1985).

Biomass of epiphytic invertebrates (milligrams per gram of plants) in each size fraction ($\log_2$ size groupings) was multiplied by total biomass of submerged macrophytes to yield grams per square metre, and this product was added to the biomass estimated for each size fraction of the sediment benthos to yield the total grams wet weight per square metre for each fraction. The sites within Lake Memphremagog were sampled monthly during August–October 1984 and May–August 1985. The sites in the other lakes were sampled monthly during May–October 1985. The estimates obtained from the six sampling times at each site were averaged to yield the seasonal mean biomass for each of the size fractions between 0.5 mg and 0.5 g.

The normalized size spectra were then determined by regressing the log [(seasonal mean biomass in size class)/(weight range of the interval)] against the log of the upper limit of the size class following Sprules and Munawar (1986) and Hanson (1990). The slopes of these regression lines can then be used to characterize biomass trends across the size spectrum, which provides a simple parameter with which the size structure of communities can be compared (Kerr 1974; Sheldon et al. 1977; Sprules and Munawar 1986). The size-spectrum parameters were calculated across size classes ranging from 0.5 mg to 5 g, which corresponds to the size range most commonly studied by benthologists and normally designated as macrozoobenthos. Although smaller size categories (down to 0.1 mg) were sampled during this study (0.2-mm screen), most authors find such fine screens too slow and prefer to pass their samples through 0.4- to 0.5-mm screens and sort by naked eye (which is inadequate for animals <1 mg) rather than by microscope.

Analysis of Littoral Habitat Conditions

At each site, slope was measured by echo-sounding along a transect perpendicular to shore, as described by Duarte and Kalff (1986). The index of exposure was calculated from 1:25 000 maps as the contour (circular) integral of the fetch ($\int F d\theta$ where F = fetch for a given angle θ). This circular integral describes the area exposed (visible) from the sampling site (Rasmussen 1988a, 1988b). Sediment water content (percent weight loss after overnight drying at 80°C), sediment organic matter content (percent weight loss on ignition at 450°C for 24 h), and E_h (oxidation–reduction potential (millivolts) using an Orion platinum electrode) were also measured at each site (Rowan et al. 1992a). Secchi disk transparency (20-cm Secchi disk), concentration of chlorophyll *a* (micrograms per litre) (spectrophotometric analysis of acetone extract), water colour (Hach colorimeter), calcium concentration (Orion calcium ion electrode), and conductivity (microsiemens at 25°C) were measured beyond the macrophyte bed at each site (methods outlined by Rasmussen 1988a, 1988b) since it was intended that they characterize the water being circulated into the littoral through wave action.

Statistical Analyses

The data set collected during this study consists of three sets of interrelated variables: (1) size structure, i.e., biomass across logarithmic size categories, (2) limnological site characteristics, and (3) macrophyte species composition (i.e., the biomass of each macrophyte species at each site). The relationships among all of these variables were first explored by PCA (SYSTAT, FACTOR, Wilkinson 1989). The relationships expressed by examining the first two principal components were then examined using log-linear least-squares multiple regression. Thus the size categories that loaded most strongly to each component were regressed against the site variables with strong loadings (+ or −) on the same components. A few of the variables tested in the multiple regressions were not statistically significant (t-values with $p > 0.05$ or failing to increase the overall F-value of the regression) and were dropped from the models. Linear regression was also used to model the relationships between size-spectrum parameters (slope and intercept of the NSD) and the limnological predictors that were found to affect the size structure. The regression coefficient of the NSD obtained for each site was regressed against site-specific estimates of total macrophyte biomass, exposure, slope, and chlorophyll *a* content of the water. All regression analyses were screened for serious multicollinearities using the condition index and the variance decomposition index provided by SYSTAT (Belsley et al. 1980; Wilkinson 1989). Logarithmic transformations were used for both independent and dependent variables because they provided good linear fits and homoscedastic residuals.

Results

A wide range of biomass size spectra was encountered in this study (Fig. 1). Most showed bimodality with biomass peaks between 1 and 4 mg and again between 64 and 256 mg; minimum biomass generally occured between 8 and 32 mg. The 1–4 mg peak mainly represented chironomid larvae, small snails, small amphipods (*Hyalella*), isopods, small trichoptera (leptocerids mainly), small mayflies (caenids, baetids, and leptophlebiids), and oligochaetes (naidids and tubificids). The trough at 8–32 mg mainly represented large amphipods (*Gammarus*), and the large size peak at 64–256 mg represented mainly odonate larvae, *Hexagenia*, lumbriculid oligochetes, leeches, and large snails.

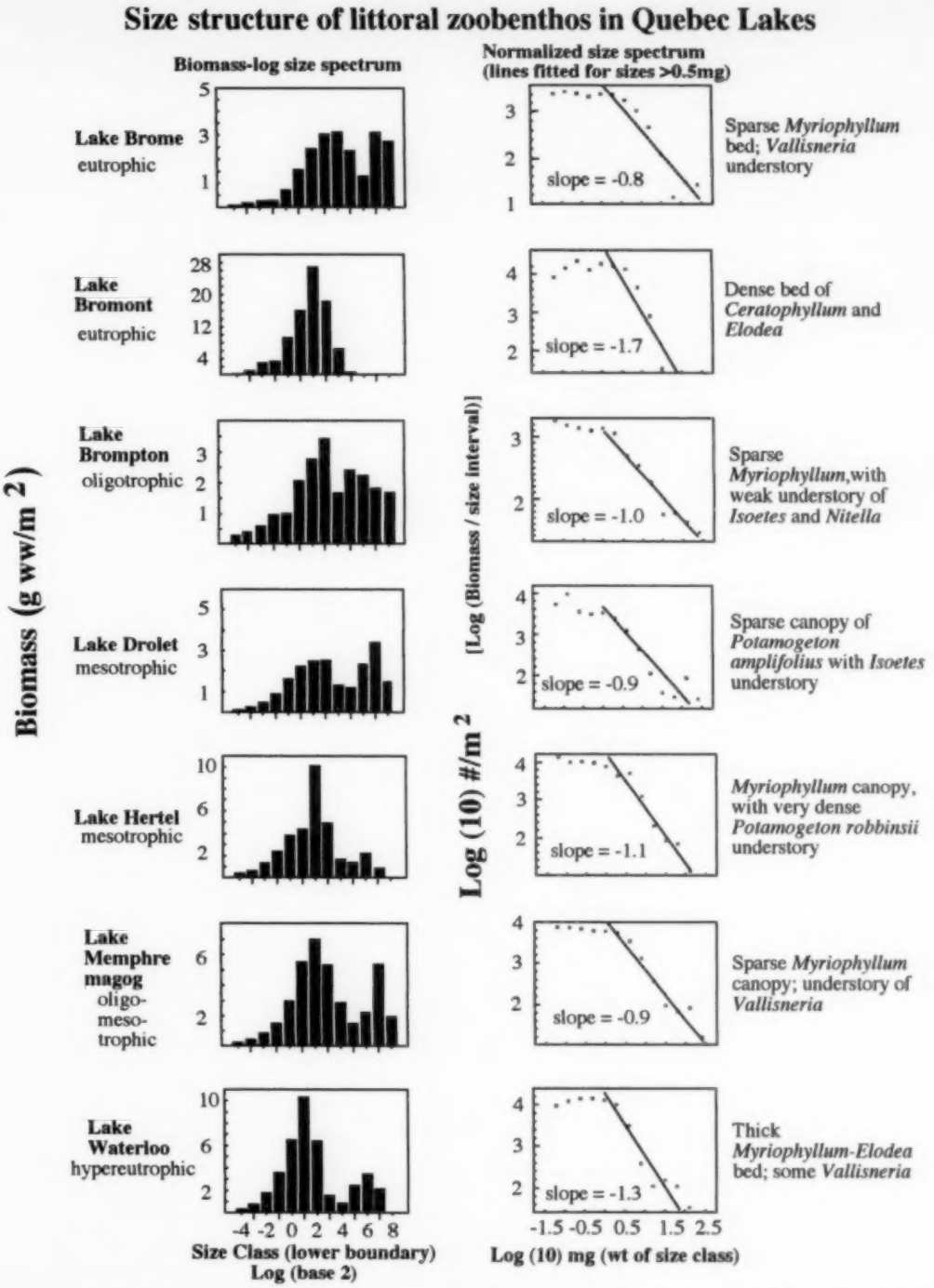

FIG. 1. A variety of representative size spectra from Lake Memphremagog and the other lakes of the study area. The left panels show the biomass–size representations, and the right panels show the NSDs whose slopes range from −0.6 to −1.7.

While in some cases the two size peaks were approximately equal in biomass, they were just as often unequally represented.

Figure 1 presents size spectra from a few of the sites studied in order to present a cross-sectional description; it shows the broad range of development of the large and small size fractions and the habitat conditions with which they were generally associated. The larger size peak contained more biomass than the small peak at open-shore sites in large lakes; in Fig. 1, a site from

Lake Brompton is shown as an example. Such sites are nondepositional (sensu Rowan et al. 1992b), wave exposure is great, sediments are coarse, and macrophyte biomass is low limited to a diffuse understory of low-growing plants (*Vallisneria, Isoetes*, and some *Najas*). At sites with low wave action, fine sediments accumulate and macrophyte biomass is highest (*Elodea* beds or *Myriophyllum spicatum* canopies with extremely dense understories of *Potamogeton robbinsii*). Here the small size peak has very high biomass, and the large size peak is often reduced in abundance or absent. In Fig. 1, sites from Lakes Hertel and Bromont are presented as examples. Many of the sites were intermediate between these two extremes, with moderate wave exposure, silty sediments, and moderate macrophyte biomass (canopy species such as *Potamogeton amplifolius* and *M. spicatum*, with mainly *Vallisneria* in the understory). Such intermediate sites had approximately equal biomass in the two benthic size fractions, and sites from Lakes Brome and Memphremagog are presented as examples in Fig. 1. Lake Waterloo was hypereutrophic, and a site from this lake illustrates the trend toward strong development of the small size fraction in the littoral zones of eutrophic lakes (Fig. 1).

While it can be seen from Fig. 1 that the biomass development of the small and large biomass peaks is highly variable, their location along the size-class axis was very consistent, and the position of the trough or low-biomass size classes was also very consistent. In fact, the positioning of both peaks and the trough did not vary by more than one size class throughout the study.

Multivariate Analyses

The statistical patterns in the data were first explored with PCA. This analysis showed clearly that the invertebrate size structure (biomass across logarithmic size categories) was functionally as well as structurally bimodal. That is, the small and large size fractions that were identified from a visual examination of the size spectra loaded to different component axes (Fig. 2a). The small invertebrate size classes (1–16 mg) tended to ordinate in the direction of increasing total macrophyte biomass, chlorophyll *a* content, water colour, sediment water content, and decreasing sediment E_h (positive direction on axis I). Canopy-forming macrophytes (mainly *Myriophyllum* and *Potamogeton crispus*) ordinated in this direction as well whereas low-growing forms such as *Nitella* and *Isoetes* ordinated in the negative direction. Large invertebrate size categories (>16 mg) loaded orthogonally to the small size fractions and were linked mainly to site exposure (wave action), with weaker loading from calcium concentration (axis II). In contrast with small invertebrates, which were positively related to canopy-forming macrophytes, large invertebrates were only well associated with understory plants. They were negatively related to *P. robbinsii*, which tends to form a densely packed understory, and positively related to *Vallisneria*, which is usually found forming a sparse understory in areas with moderate wave action. These multivariate relationships are displayed as a bivariate plot based on the loadings of each variable to the first two components obtained by factoring the correlation matrix of the entire data set (SYSTAT, FACTOR). The size-class loadings were also calculated by factoring a correlation matrix of size classes only, and these were almost indentical to those obtained from the entire matrix (Fig. 2a). Components I and II derived from the size-class correlation matrix explained 81% of the variance in size-class data matrix (Tables 1–3).

Multiple Regressions Predicting the Abundance of Small and Large Invertebrates

Multiple regression analyses carried out on the two different size fractions further supported the interpretation that the two size modes represent independent and functionally distinct components of the benthic community. The two size classes were not correlated (neither univariate nor partially) and were associated with different limnological variables and with different macrophyte communities. The biomass of small invertebrates is strongly linked to plant biomass (+) and to trophic status (+) as indicated by the chlorophyll *a* content of the water ($R^2 = 0.84$) (Table 4, Eq. 2, Fig. 3a). The relationship obtained in Lake Memphremagog was not significantly different from that obtained in other lakes, since a categorical variable (Lake Memphremagog = 1, other sites = 0) made no significant contribution to the model when tested both directly and for its interaction with plant biomass in Eq. 1–3 (Fig. 3a). In predicting the biomass of the small size fraction, the slope of the littoral shore was always statistically significant when substituted for plant biomass, and the Secchi disk transparency was significant when substituted for chlorophyll *a* (Table 4, Eq. 3). The biomass of large invertebrates showed a weak parabolic regression with exposure ($R^2 = 0.36$, $p < 0.001$)(Table 4, Eq. 4) and a somewhat stronger regression which also included the littoral slope (−) and the water colour (−) ($R^2 = 0.47$)(Table 4, Eq. 5). Since most of the sites with high wave exposure were in Lake Memphremagog, and it is not possible to find sites in Lake Memphremagog comparable in exposure with those of the smallest lakes (Fig. 3b), it is not possible to test whether the relationship between large invertebrate biomass and wave exposure is the same in Lake Memphremagog as in the other lakes.

Size-Spectrum Parameters

The slopes of the NSD ranged from −0.6 to −1.7, and this variation corresponded mainly to variability in the relative abundance of the two size peaks of benthic invertebrates (Tables 2 and 4, Eq. 6–8). The slope of the NSD becomes steeper (more negative) as the biomass in the 1–16 mg size range increases relative to that in the 16–500 mg size range. When biomass tends to increase with size, NSD slopes are greater than −1.0, and when biomass tends to decrease with size, slopes are less than −1.0. Intercepts of the NSD range from 0.2 to 1.4 and are strongly negatively correlated with their slopes (Table 5, Eq. 14); that is, when slopes are steep (more negative), intercepts (estimated biomass for log size = 0) are high. The prediction of size-spectra parameters was in all cases significantly improved by site exposure and by the chlorophyll *a* concentration of the water. NSD slope increased (became less negative) and the intercept decreased with increasing exposure; this reflects the increased prominence of the large animal size fraction relative to that of the small animals with larger wave exposure. NSD slope decreased (became more negative) and its intercept increased with increasing lake trophic status (chlorophyll *a* concentration); thus, increased trophic status increases the relative prominence of the small animal size fraction. An ANCOVA analysis using a categorical variable showed that Lake Memphremagog was not different from the other lakes with regard to the relationship predicting NSD slope from plant biomass, chlorophyll *a*, and exposure (Table 5, Eq. 10).

TABLE 1. Size structure (g wet weight/m^2, seasonal average) of littoral zoobenthos in some Quebec Eastern Townships lakes.

		Size class (mg)												
													Large	Small
Lake	Site	0.5–1	1–2	2–4	4–8	8–16	16–32	32–64	64–128	128–256	256–512	Total	(16–512)	(0.5–16)
Silver	1	1.0	1.1	1.1	1.1	0.7	0.9	1.2	1.5	4.2	5.0	18.7	12.8	4.9
	2	1.1	1.2	1.2	1.2	0.8	0.9	1.3	1.5	4.3	5.2	20.1	13.2	5.6
	3	0.7	0.8	0.8	0.8	0.6	0.5	0.7	0.8	2.3	2.8	10.2	7.1	3.7
	4	0.3	0.3	0.3	0.3	0.2	0.4	0.6	0.7	2.0	2.4	7.5	6.1	1.5
D'argent	1	0.1	0.5	0.8	1.0	0.7	1.2	1.0	0.2	1.1	2.6	9.3	6.2	3.1
	2	0.5	1.6	2.6	3.4	2.5	0.8	0.7	0.1	0.8	1.7	14.7	4.1	10.5
	3	0.5	1.8	3.0	4.0	2.8	1.4	1.2	0.3	1.3	3.1	20.3	7.3	12.1
Waterloo	1	4.1	7.4	11.8	7.3	1.8	1.0	2.6	3.8	2.3	0.0	42.8	9.7	32.5
	2	3.5	6.5	10.3	6.4	1.6	0.8	2.2	3.1	1.9	0.0	36.8	8.1	28.2
	3	4.7	8.6	13.7	8.5	2.1	0.5	1.3	1.9	1.2	0.0	43.4	4.9	37.5
Roxton	1	2.0	4.3	5.9	5.1	2.4	1.6	0.0	0.0	0.0	0.0	21.8	1.6	19.7
Brompton	1	0.4	1.0	2.1	2.7	3.6	1.7	2.1	2.2	1.7	1.6	19.5	9.3	9.9
	2	0.1	0.3	0.6	0.8	1.1	2.3	2.8	2.8	2.2	2.1	15.4	12.1	3.0
Brome	1	0.5	1.7	3.9	5.8	7.0	4.3	3.3	2.1	4.3	4.0	36.7	18.0	18.8
	2	0.2	0.7	1.6	2.4	2.9	2.9	2.3	1.4	2.9	2.7	19.9	12.2	7.8
Drolet	1	0.6	1.3	2.0	2.6	2.6	1.3	1.1	2.4	3.1	1.7	19.1	9.6	9.1
	2	1.2	2.6	4.1	5.3	5.3	1.2	1.1	2.3	2.9	1.6	27.1	9.1	18.5
Magog	1	6.6	12.4	15.5	14.0	6.0	0.9	1.5	3.1	2.2	0.0	62.6	7.8	54.6
	2	2.3	4.3	5.4	4.9	2.1	0.9	1.4	2.9	2.1	0.0	26.5	7.2	18.9
	3	1.5	2.8	3.4	3.1	1.3	1.5	2.3	4.9	3.5	0.0	24.1	12.1	12.1
Hertel	1	3.5	5.6	6.3	14.7	7.1	1.3	1.1	1.7	0.7	0.0	42.5	4.8	37.2
	2	1.3	2.1	2.3	5.4	2.6	1.3	1.0	1.7	0.6	0.0	18.4	4.6	13.7
	3	2.9	4.7	5.3	12.3	6.0	1.3	1.1	1.7	0.7	0.0	36.1	4.8	31.1
Bromont	1	3.5	10.0	17.5	29.5	19.1	6.4	0.5	0.0	0.0	0.0	85.6	7.0	79.6
Memphremagog														
Newport Bay	1	4.6	13.3	25.0	31.4	23.7	3.8	2.0	3.0	7.1	2.6	118.5	18.6	98.2
	2	1.5	4.4	8.3	10.4	7.9	6.8	3.6	5.3	12.7	4.7	66.0	33.2	32.6
	3	2.3	6.8	12.7	16.0	12.2	2.7	1.4	2.1	5.0	1.9	67.3	13.2	50.0
South Basin	1	0.1	0.4	0.8	1.0	0.7	2.6	1.4	2.0	4.8	1.8	15.8	12.5	3.1
	2	1.4	4.0	7.5	9.5	7.2	2.1	1.1	1.6	3.9	1.4	40.0	10.1	29.6
	3	1.0	2.9	5.5	6.9	5.2	2.9	1.5	2.2	5.3	2.0	37.3	14.1	21.5
	4	1.3	3.7	6.9	8.6	6.5	1.5	0.8	1.1	2.7	1.0	34.9	7.1	27.0
	5	0.1	0.2	0.4	0.5	0.4	2.0	1.1	1.6	3.8	1.4	11.2	9.8	1.6
	6	1.4	4.2	7.8	9.9	7.4	5.3	2.8	4.1	9.8	3.7	53.4	25.6	30.8
	7	0.6	1.8	3.4	4.3	3.2	2.5	1.3	1.9	4.6	1.7	26.0	12.0	13.5
	8	0.1	0.3	0.6	0.8	0.6	1.9	1.0	1. 4	3.5	1.3	11.3	9.0	2.6
Central Basin	1	0.9	2.6	4.8	6.1	4.6	4.0	2.2	3.1	7.5	2.8	37.3	19.7	18.9
	2	0.3	0.8	1.5	1.8	1.4	2.0	1.1	1.5	3.7	1.4	12.9	9.7	5.7
	3	1.0	3.0	5.5	7.0	5.3	2.9	1.5	2.2	5.3	2.0	36.1	13.9	21.8
	4	1.8	5.3	9.9	12.5	9.5	1.6	0.8	1. 2	2.7	1.1	47.2	7.5	39.1
	5	0.4	1.2	2.2	2.8	2.1	3.4	1.8	2.6	6.3	2.3	25.5	16.4	8.6
	6	0.5	1.5	2.8	3.5	2.6	1.4	0.7	1.1	2.6	1.0	18.3	6.7	11.1
Fitch Bay	1	0.4	1.0	2.0	2.5	1.9	4.5	2.4	3.5	8.5	3.1	29.0	22.1	7.7
	2	0.7	2.2	4.0	5.1	3.8	3.9	2.1	3.0	7.3	2.7	35.4	18.9	15.9
	3	0.7	2.0	3.7	4.7	3.5	4.8	2.6	3.7	9.0	3.3	37.0	23.5	14.6
Sargent's Bay	1	1.4	4.1	7.6	9.6	7.2	2.4	1.3	1.9	4.5	1.6	43.3	11.8	29.9
	2	0.2	0.7	1.3	1.6	1.2	1.2	0.7	1.0	2.3	0.9	11.3	6.0	5.1
North Basin	1	0.4	1.3	2.5	3.1	2.3	2.5	1.3	1.9	4.6	1.7	22.0	12.1	9.7
	2	0.6	1.8	3.5	4.3	3.3	2.9	1.6	2.3	5.5	2.0	27.8	14.3	13.6
	3	0.3	0.8	1.6	1.9	1.5	2.5	1.4	2.0	4.7	1.8	17.5	12.3	6.1
	4	0.3	1.0	1.8	2.3	1.8	1.4	0.8	1.1	2.7	1.0	14.6	7.0	7.3
	5	0.5	1.6	3.0	3.8	2.8	2.7	1.4	2.1	5.1	1.9	25.1	13.2	11.8

TABLE 1. (*Concluded*)

Lake	Site	Size class (mg) 0.5–1	1–2	2–4	4–8	8–16	16–32	32–64	64–128	128–256	256–512	Total	Large (16–512)	Small (0.5–16)
Channel Bay	1	1.0	2.9	5.4	6.8	5.1	7.2	3.9	5.6	13.5	5.0	52.2	35.3	21.2
	2	0.2	0.6	1.1	1.3	1.0	2.9	1.5	2.2	5.3	2.0	15.2	13.9	4.1
Memphremagog														
Average		0.9	2.6	4.9	6.3	5.1	4.0	3.7	6.5	14.0	19.1	34.1	14.8	19.4
SD		0.9	2.5	4.8	6.0	5.0	5.4	11.2	22.6	45.0	91.5	22.2	7.4	19.4
Other lakes														
Average		1.8	3.4	5.0	6.0	4.0	2.8	3.9	6.8	12.2	21.9	28.3	8.3	19.7
SD		1.7	3.2	4.8	6.2	4.5	6.1	12.3	24.8	49.8	100.0	17.6	3.7	18.6

TABLE 2. Parameters of the NSD (intercept and slope), together with morphometric and physicochemical site characteristics.

Lake	Site	NSD Intercept	NSD Slope	Sample Depth (m)	Ca (mg/L)	Plant biomass (kg/m^2)	Littoral slope (%)	Exposure (km^2)	E_h (mV)	Chl a (µg/L)	Secchi depth (m)
Silver	1	0.2	−0.7	4.0	9.0	0.0	12.0	0.7	−117	3.5	5.8
	2	1.1	−1.2	2.0	9.0	0.6	2.2	0.7	−175	3.5	5.8
	3	0.3	−0.7	4.0	9.0	0.0	14.0	0.5	−122	3.5	5.8
	4	0.1	−0.6	4.0	9.0	0.0	25.0	0.7	−45	3.5	5.8
D'argent	1	0.2	−0.7	1.8	7.6	0.0	18.0	0.9	−65	3.5	2.6
	2	0.9	−1.2	2.0	7.6	0.5	6.0	0.9	−73	3.5	2.6
	3	0.6	−1.1	2.0	7.6	0.4	1.0	0.9	−61	3.5	2.6
Waterloo	1	1.1	−1.3	1.2	14.0	1.3	8.6	1.3	−280	24.0	0.9
	2	1.1	−1.3	1.0	14.0	1.2	0.9	1.3	−264	24.0	0.9
	3	1.3	−1.5	1.0	14.0	1.4	0.4	0.1	−295	24.0	0.9
Roxton	1	1.2	−1.7	2.2	9.4	1.3	0.8	1.7	−220	8.5	2.3
Brompton	1	0.6	−1.1	1.5	4.1	0.5	3.0	2.2	−72	1.3	4.2
	2	0.3	−0.7	2.1	4.1	0.1	7.0	11.2	−38	1.3	4.2
Brome	1	0.7	−1.0	1.8	9.3	0.3	0.6	6.5	−112	10.1	2.8
	2	0.4	−0.8	2.4	9.3	0.1	5.0	13.6	−104	10.1	2.8
Drolet	1	0.5	−0.9	2.3	4.0	0.1	5.0	2.1	5	5.5	3.8
	2	0.8	−1.1	2.4	4.0	0.3	0.5	2.1	−152	5.5	3.8
Magog	1	1.2	−1.5	1.8	15.4	1.6	0.5	1.8	−265	11.0	2.3
	2	1.0	−1.2	2.3	15.4	0.8	5.2	10.1	−163	11.0	2.3
	3	0.6	−1.0	2.3	15.4	0.3	9.0	10.1	−122	11.0	2.3
Hertel	1	1.1	−1.5	3.5	12.0	3.1	5.1	0.3	−210	3.0	3.5
	2	1.0	−1.2	3.3	12.0	0.8	11.0	0.3	−196	3.0	3.5
	3	1.2	−1.4	3.8	12.0	2.5	5.0	0.3	−206	3.0	3.5
Bromont	1	1.7	−1.7	1.6	10.0	2.9	1.0	0.2	−210	9.6	2.1
Memphremagog											
Newport Bay	1	1.4	−1.2	2.5	18.0	1.9	0.2	3.1	−230	12.0	2.5
	2	0.6	−1.0	3.1	18.0	0.8	1.0	5.8	−154	10.4	3.0
	3	1.3	−1.3	4.2	18.0	1.5	2.0	4.1	−218	10.4	2.9
South Basin	1	0.2	−0.7	4.2	18.5	0.1	20.0	22.5	70	6.2	3.5
	2	0.8	−1.2	2.5	18.5	1.2	1.1	1.9	−175	6.0	3.8
	3	0.8	−0.9	3.0	18.5	0.6	5.0	20.6	−80	6.2	3.8
	4	1.0	−1.1	3.5	18.5	0.6	1.7	12.3	−121	6.0	3.3
	5	−0.1	−0.6	3.0	18.5	0.1	18.0	14.5	69	6.1	3.3
	6	1.0	−1.0	2.6	18.5	0.7	1.5	16.2	−82	6.8	3.4
	7	0.6	−0.9	2.5	18.5	0.7	4.0	35.8	−106	6.0	3.6
	8	0.2	−0.7	4.5	18.5	0.1	18.0	17.2	42	5.5	3.8

TABLE 2. (*Concluded*)

Lake	Site	NSD Intercept	NSD Slope	Sample Depth (m)	Ca (mg/L)	Plant biomass (kg/m^2)	Littoral slope (%)	Exposure (km^2)	E_h (mV)	Chl a (µg/L)	Secchi depth (m)
Central Basin	1	0.6	−1.0	3.0	18.5	0.6	5.0	20.6	−80	6.2	3.8
	2	0.0	−0.6	3.8	17.3	0.1	11.0	24.4	−78	4.5	4.3
	3	1.0	−1.1	2.5	18.0	0.8	0.9	3.8	−125	3.5	4.2
	4	1.1	−1.2	3.5	17.3	1.7	0.3	4.8	−195	4.5	4.2
	5	0.5	−0.8	3.2	17.3	0.5	6.6	12.8	−165	3.5	4.8
	6	0.6	−1.0	4.0	17.3	0.4	5.4	16.9	−13	3.4	4.9
Fitch Bay	1	0.5	−0.8	3.5	15.5	0.2	14.0	6.7	−88	5.5	3.8
	2	0.6	−1.0	2.5	15.5	0.8	2.1	2.6	−112	3.2	3.6
	3	0.4	−0.9	1.5	15.5	0.5	3.8	3.7	−62	3.6	3.6
Sargent's Bay	1	1.1	−1.2	2.9	17.3	1.6	1.8	2.1	−155	3.0	5.0
	2	0.0	−0.7	2.2	17.3	0.1	19.0	2.0	−24	3.0	5.0
North Basin	1	0.4	−0.8	2.8	18.0	0.4	1.5	10.8	−74	3.1	4.9
	2	0.7	−0.9	3.2	18.0	0.7	4.2	3.2	−87	3.0	4.9
	3	0.3	−0.8	3.5	18.0	0.4	3.5	11.9	−64	3.0	5.3
	4	0.3	−0.8	3.2	18.0	0.3	8.0	10.9	35	3.1	5.0
	5	0.3	−1.0	2.5	18.0	0.5	2.0	7.4	−135	3.5	5.3
Channel Bay	1	0.5	−1.0	2.5	18.0	1.1	4.0	5.1	−83	4.1	5.1
	2	0.1	−0.7	3.7	18.0	0.3	3.0	6.2	10	3.2	5.2
Memphremagog											
Average		0.6	−0.9	3.1	17.8	0.7	5.8	10.7	−86	5.1	4.1
SD		0.4	0.2	0.7	0.9	0.5	6.1	8.4	80	2.4	0.8
Other lakes											
Average		0.8	−1.1	2.3	9.9	0.8	6.1	2.9	−148	7.9	3.2
SD		0.4	0.3	0.9	3.6	0.9	6.2	4.1	84	6.9	1.5

TABLE 3. Species[a] breakdown of aquatic macrophyte biomass (g wet weight/m^2, seasonal average) at each sampling site.

Lake	MSPIC	PPERF	PCRISP	PAMP	ELODEA	CERAT	HETER	PROB	VALLIS	NAJ	NIT	ISOET	Total
Silver	0	0	0	0	0	0	0	5	0	4	6	15	30
	0	0	0	0	620	0	0	12	0	8	0	0	640
	0	0	0	0	0	0	0	10	0	2	8	12	32
	0	0	0	0	0	0	0	4	0	1	1	6	12
D'argent	0	0	0	0	0	0	0	0	8	3	2	7	20
	235	50	0	200	0	0	0	0	21	6	8	0	520
	95	30	0	195	0	0	0	0	18	7	5	0	350
Waterloo	950	0	0	0	150	0	0	0	220	0	0	0	1320
	910	0	0	0	145	0	0	0	165	0	0	0	1220
	980	0	0	0	220	0	0	0	190	0	0	0	1390
Roxton	1100	0	0	0	0	0	200	0	0	0	0	0	1300
Brompton	30	20	0	315	0	0	0	0	80	5	0	0	450
	0	0	20	0	0	0	0	0	7	6	12	35	80
Brome	85	25	0	60	0	0	0	0	62	10	3	35	280
	0	0	0	0	0	0	0	0	77	8	5	0	90
Drolet	0	0	0	0	0	0	0	0	0	4	20	106	130
	0	0	0	190	0	0	0	0	0	5	25	120	340
Magog	310	0	0	0	900	200	0	180	0	0	0	0	1590
	240	0	130	0	180	0	0	260	0	20	0	0	830
	75	0	0	0	0	0	0	50	100	15	10	0	250
Hertel	165	45	40	0	0	0	0	2800	0	0	0	0	3050
	30	10	10	0	0	0	0	650	80	0	0	0	780
	110	25	25	0	0	0	0	2300	0	0	0	0	2460

TABLE 3. (*Concluded*)

Lake	MSPIC	PPERF	PCRISP	PAMP	ELODEA	CERAT	HETER	PROB	VALLIS	NAJ	NIT	ISOET	Total
Bromont	110	0	0	0	220	2210	0	320	15	25	0	0	2900
Memphremagog													
Newport Bay	930	0	720	0	200	0	0	0	50	0	0	0	1900
	635	0	0	0	0	0	0	0	110	5	0	0	750
	320	0	0	0	1200	0	0	0	0	0	0	0	1520
South Basin	0	0	0	0	0	0	0	0	35	10	5	0	50
	415	0	0	0	235	0	200	235	115	0	0	0	1200
	7	0	0	0	0	0	110	0	420	15	0	0	552
	215	0	0	0	0	0	40	0	375	0	0	0	630
	30	0	0	0	0	0	0	0	12	4	1	0	47
	250	0	0	0	0	0	85	0	345	0	0	0	680
	190	140	0	0	0	0	177	0	210	20	2	0	739
	0	0	0	0	0	0	0	0	55	15	10	0	80
Central Basin	175	45	0	0	0	0	52	0	245	28	5	0	550
	0	0	0	0	0	0	22	0	60	10	7	0	99
	305	15	15	30	0	0	45	70	300	16	4	0	800
	850	0	0	0	800	0	0	0	0	0	0	0	1650
	245	0	0	0	0	0	65	55	90	6	8	0	469
	285	10	0	0	0	0	11	0	25	9	11	0	351
Fitch Bay	105	30	0	65	0	0	0	5	10	2	2	0	219
	755	0	0	0	0	0	30	1	12	4	6	0	808
	265	0	0	0	0	0	20	0	190	20	15	0	510
Sargent's Bay	1285	0	0	0	0	0	80	26	200	6	4	0	1601
	0	0	0	45	0	0	0	0	30	18	12	0	105
North Basin	25	0	0	0	0	0	85	235	95	0	0	0	440
	322	0	0	0	0	0	25	270	120	0	0	0	737
	195	25	0	0	0	0	50	110	10	0	0	0	390
	145	0	0	0	0	0	25	55	45	12	8	0	290
	120	0	0	95	0	0	0	235	10	0	0	0	460
Channel Bay	980	0	0	0	0	0	50	25	2	3	2	0	1062
	0	0	0	0	0	0	0	250	20	0	0	0	270
Memphremagog													
Average	312	9	25	8	84	0	40	54	110	7	4	0	654
SD	343	27	133	22	261	0	51	91	121	8	4	0	498
Other lakes													
Average	226	9	9	40	101	100	8	274	44	5	4	14	836
SD	359	15	27	86	216	442	40	706	65	7	7	31	890

[a]MSPIC, *M. spicatum*; PPERF, *Potamogeton*; PCRISP, *P. crispus*; PAMP, *P. amplifolius*; ELODEA, *Elodea canadensis*; CERAT, *Ceratophyllum*; HETER, *Heteranthea dubium*; PROB, *P. robbinsii*; VALLIS, *Vallisneria*; NAJ, *Najas flexilis*; NIT, *Nitella*; ISOET, *Isoetes*.

Relationship of Invertebrate Size Structure to the Composition of Aquatic Macrophyte Communities

The hypothesis that macroinvertebrate size structure will be influenced by macrophyte species composition (Hanson 1990) was examined using the multivariate analysis shown in Fig. 2b. The strongest pattern exhibited by this analysis was the positive loading of *M. spicatum* (the main canopy-forming plant) to component I (associated with the small invertebrate size fraction. Because *Myriophyllum* frequently dominated the canopy, this observation is consistent with the previously described patterns relating the abundance of this invertebrate size fraction to total macrophyte biomass. The multivariate analysis also yielded a second, although weaker, relationship between the abundance of the larger invertebrate size fraction and the type of understory community present. This pattern is displayed mainly along component II. *Potamogeton robbinsii*, which forms a very dense understory when abundant, loads negatively, and *Vallisneria americana*, which never forms dense mats even when abundant, loads positively on component II. Loose understories are usually associated with conditions of high wave exposure (tending to high E_h and sediment with low water content), and thus the inclusion of either E_h, any of the understory species, or sediment water content adds nothing significant to the regression relating large invertebrate abundance to wave exposure (Table 4). Although patterns exist in the relationship between invertebrate size structure and macrophyte community composition, the statistical relationships to limnological variables are stronger, and there appears to be little statistical gain from the consideration of macrophyte species composition where these limnological variables are known.

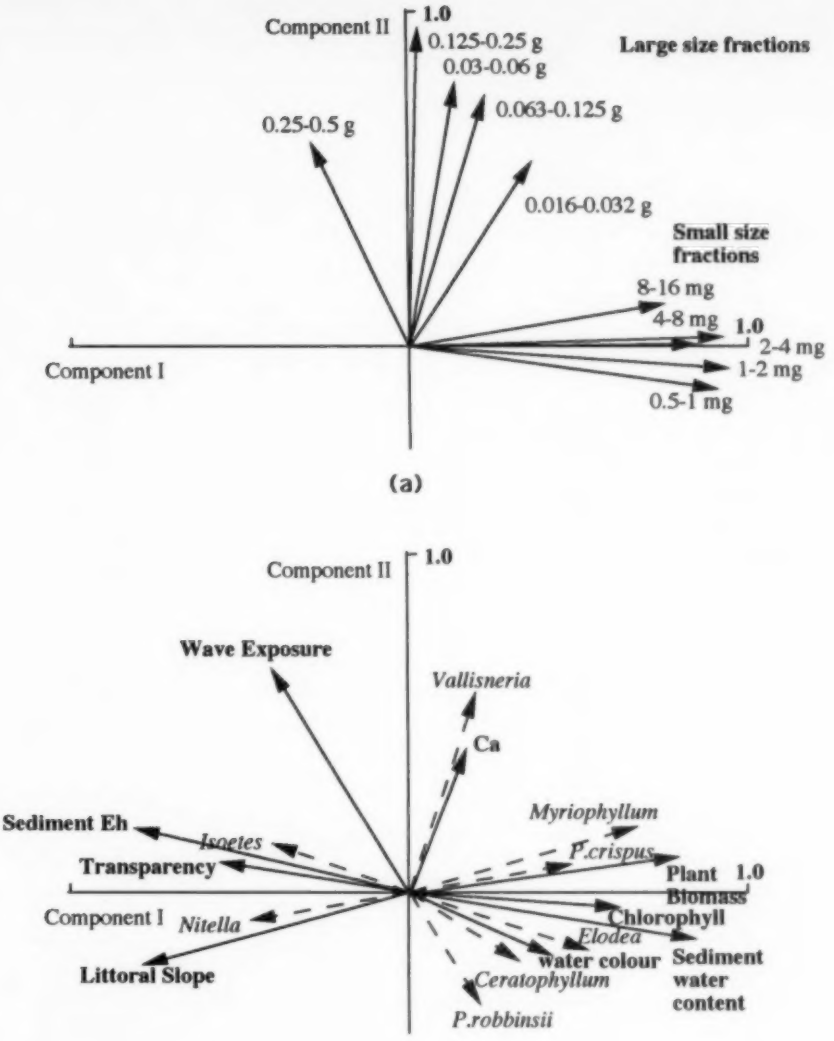

FIG. 2. Principal component ordination of biomass–size spectra. (a) Loadings of the different size fractions to the two main principal components, which together accounted for 81% of the data variance in Table 1; (b) loadings of the limnological site variables (solid lines) and macrophyte species (broken lines) to components I and II.

Discussion

Empirical Patterns in Spectral Parameters

Sprules and Munawar (1986) attempted to correlate slopes of normalized plankton size spectra with lake productivity, size, and perturbation level. They found that NSD slope decreased (became less negative) with increasing system productivity and with increasing system disturbance. More productive systems tended to exhibit greater biomass in the larger planktonic size classes (large zooplankton), although they differed little in the abundance of the smaller plankton size classes (small phytoplankton). Thus, they inferred that system enrichment was rapidly transmitted up through the small size classes and resulted in the accumulation of larger biomass in the larger size classes. The pattern in relation to trophic status was quite weak and may have had as much to do with differences in food web structure, such as the absence of mysid zooplanktivores in the productive systems (the western basin of Lake Erie and Lake St. Clair) compared with high mysid abundance in the deeper, less productive Great Lakes. Direct comparisons between size-spectrum parameters for planktonic and benthic size spectra are complicated by differences in dimensionality and boundedness. That is, pelagic systems are much more three-dimensional than

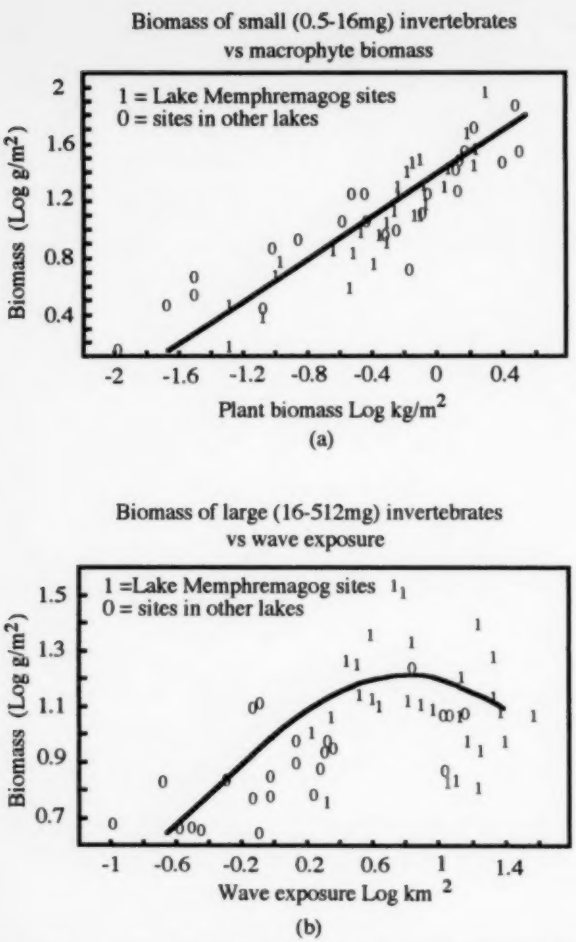

FIG. 3. Littoral macroinvertebrate size structure. (a) Relationship of the small (0.5–16 mg) size fraction to macrophyte biomass (Eq. 1); (b) relationship of the large (16 mg) size fraction to wave exposure (Eq. 4).

benthic systems and benthic systems represent sediment accumulation boundaries whereas pelagic systems are more or less unbounded. These differences have many effects on ecosystem function, although their effects on size structure are unknown. In my analysis the NSD slope became more steep (more negative) with increasing lake productivity, a pattern opposite to that described by Sprules and Munawar (1986) for lake plankton communities. Thus, there would appear to be no general pattern relating the slope of the NSD (i.e., the biomass trends across the size spectrum) to trophic status that is valid for both pelagic and benthic systems.

The Problem of Autocorrelated Residuals

The problem of comparing parameters is complicated by the peaks and troughs that appear in most size spectra. Thus the trends indentified along the spectrum will be sensitive to the overall size range chosen for a particular study, especially if peaks or troughs occur near the upper and lower boundaries of the size range investigated. The peaks and troughs in planktonic size spectra may, in fact, reflect independent groupings similar to those found in this analysis. Tessier and Horwitz (1990) found three orthogonal size fractions in lake zooplankton communities and showed that the largest fraction was strongly linked to lake alkalinity. As is the case when fitting trends to time series (Wilkinson 1989), the fitting of size-structure parameters presents problems of a purely statistical nature because the residuals (deviations of individual size-class abundances from the main linear trend) are usually very autocorrelated. This can be seen in the benthic size spectra from this study (Fig. 1) (see also Hanson et al. 1989; Hanson 1990), as well as those fitted to pelagic plankton communities by Sprules and Munawar (1986) and Sprules et al. (1991). With such peaks and troughs in the spectra, parameter estimates for the NSD or the biomass versus size spectrum are not statistically robust. That is, they will be highly sensitive to the positioning of peaks and troughs in relation to the upper and lower size boundaries, which are arbitrary limits chosen differently for different studies. As one of the main goals in size-structure modelling is to predict abundances of difficult-to-sample size classes from more workable parts of the spectrum, this is likely a serious limitation of the approach.

Two possible approaches can be chosen to deal with the "lack of fit" problems presented by trended (positively autocorrelated) residuals. One common solution is to fit more parameters to improve the statistical fit of the data to the underlying trend, and another is to integrate across the peaks, using the troughs as boundaries. Adding parameters improves fit at the cost of a bewildering array of strongly interdependent parameters that are difficult to estimate robustly, interpret, and compare. Thus, the approach of fitting more parameters merely transfers the problem of autocorrelation (lack of independence) from the size classes themselves to the parameters of the model. It is therefore merely a cosmetic solution that further compounds the problem.

A. Morin (personal communication) suggested that although the NSD slope is an unreliable parameter due to the presence of peaks and troughs, the NSD would provide a better index of departure from uniform biomass if one were to calculate the residual variance of the NSD with respect to a line of slope −1 forced through the data. I tested this on the data set by calculating the cumulative departure from uniform biomass (Σ (observed − mean biomass)^2 over the 0.5–500 mg size range) for each of the 53 study sites and testing this parameter (VAR) against my environmental variables. The mean biomass used was not the global mean for the whole data set, but rather the site mean obtained by averaging biomass over the size classes. VAR was significantly correlated with the NSD slope ($r = -0.70$) and was strongly related to plant biomass and chlorophyll *a*, the two best predictors of the NSD slope. The relationship obtained for the 53 sites was

$$\log \mathrm{VAR} = 0.83 + 0.62 \log \text{plant biomass} + 0.03 \log \mathrm{Chl}\,a \quad (15)$$

$$R^2 = 0.76,\ \mathrm{SE}_{\mathrm{est}} = 0.29.$$

Although VAR is likely a less biased descriptor of departure from uniform biomass than the slope of the NSD, the two parameters behave quite similarly with respect to the environmental variables in this data set.

Because the positioning of peaks and troughs within the size spectrum tends to be highly repeatable across sampling sites (Fig. 1), integrated biomass estimates for the particular size categories defined by these peaks should provide consistent and

TABLE 4. Regression models involving orthogonal size fractions.

(a) Models predicting the small (0.5–16 mg) and large (16–512 mg) size fractions from environmental variables:

(1) log small fraction (SF) (g/m^2) = 1.37 + 0.64 (±0.047) log macrophyte biomass (kg/m^2); $R^2 = 0.79$, $SE_{est} = 0.19$

(2) log SF = 1.09 + 0.59 (±0.04) log macrophyte biomass + 0.37 (±0.09) log chlorophyll *a*; $R^2 = 0.84$, $SE_{est} = 0.17$

(3) log SF = 1.73 − 0.52 (±0.07) log littoral slope − 0.10 (±0.03) log transparency; $R^2 = 0.63$, $SE_{est} = 0.26$

(4) log large fraction (LF) = 0.97 + 0.30 (±0.06) EXP − 0.16 (±0.06) EXP^2 where EXP = log exposure; $R^2 = 0.36$, $SE_{est} = 0.19$

(5) log LF = 1.13 + 0.29 (±0.06) EXP − 0.14 (±0.06) EXP^2 − 0.14 (±0.05) log littoral slope − 0.13 (±0.06) log colour; $R^2 = 0.47$, $SE_{est} = 0.17$

(b) Relationships between orthogonal size fractions and the NSD parameters:

(6) NSD slope = −0.90 − 0.55 (±0.036) log SF + 0.49 (±0.06) log LF; $R^2 = 0.85$, $SE_{est} = 0.11$

(7) NSD intercept = 0.14 + 0.89 (±0.06) log SF − 0.45 (±0.11) log LF; $R^2 = 0.82$, $SE_{est} = 0.19$

(8) NSD slope = −0.96 − 0.54 (±0.03) SF/LF; $R^2 = 0.85$, $SE_{est} = 0.11$

TABLE 5. Regression models predicting size parameters from trophic and morphometric variables.

(a) Predicting the parameters of the NSD from environmental variables:

(9) NSD slope = −1.17 − 0.39 (±0.04) log macrophyte biomass (kg/m^2); $R^2 = 0.68$, $SE_{est} = 0.16$

(10) NSD slope = −1.11 − 0.33 (±0.03) log macrophyte biomass − 0.19 (±0.06) log chlorophyll *a* (μg/L) + 0.18 (±0.025) log exposure (km^2); $R^2 = 0.85$, $SE_{est} = 0.11$

(11) NSD intercept = 0.73 + 0.51 (±0.05) log macrophyte biomass + 0.37 (±0.09) log chlorophyll *a* − 0.21 (±0.04) log exposure; $R^2 = 0.82$, $SE_{est} = 0.18$

(b) Models including littoral slope and Secchi transparency in place of macrophyte biomass and chlorophyll *a*:

(12) NSD slope = −1.50 + 0.25 (±0.04) log littoral slope (%) + 0.07 (±0.02) log Secchi transparency (m) + 0.18 (±0.036) log exposure; $R^2 = 0.69$, $SE_{est} = 0.16$

(13) NSD intercept = 1.40 − 0.40 (±0.07) log littoral slope (%) − 0.11 (±0.03) log Secchi transparency (m) − 0.21 (±0.06) log exposure; $R^2 = 0.64$, $SE_{est} = 0.26$

Parameters of the NSD are strongly related to each other:

(14) NSD slope = −0.61 − 0.60 (±0.03) NSD intercept; $R^2 = 0.87$, $SE_{est} = 0.10$

interpretable parameters. Indeed the multivariate analyses of size structure (Fig. 2) verified this and demonstrated that the two size peaks of zoobenthos from my study lakes were largely independent of each other and linked to different environmental factors. The smaller biomass peak was highly predictable from the macrophyte biomass and the trophic status of the lake whereas the larger biomass peak exhibited a somewhat weaker trend with exposure to waves (Fig. 3; Table 4).

The connection between exposure and large invertebrate biomass suggests that physical energy inputs may enhance benthic production as postulated by Nixon (1988) to account for coastal marine macrobenthic biomass being higher than that of lakes. Wave energy may enhance benthic productivity in a number of ways: (1) through sediment mixing leading to more efficient processing of sediment organic matter, (2) enhanced oxygen supply and reduced stagnation at the sediment–water interface leading to higher sediment E_h (see Rasmussen 1988b), or (3) enhanced flux of fresh seston to bottom communities. The parabolic relationship between the biomass of the large size fraction and exposure suggests that while physical energy input may be on the whole stimulatory, too much physical energy may have adverse effects, perhaps by reducing the amount of fine

particulate matter in the sediment profile. Rowan et al. (1992a, 1992b) presented models showing how high exposure (wave energy) can raise the water velocity at the sediment–water interface above the threshold for fine particle accumulation in a manner that interacts with depth and slope.

My analysis showed that the strong empirical regressions linking the slope of the NSD across the 0.5 mg to 0.5 g size range was mostly determined by the explainable variation of the small size fraction, and that variation in the biomass of large size classes contributed less to the variation in the slope or intercept of the NSD. Parameters obtained by fitting NSDs to size spectra do not allow detection of functionally independent size fractions, and thus, PCA analysis of size structure can complement the NSD approach in an important way. However, it is not known whether peaks in size spectra correspond in general to independent size fractions; in fact, this question could be pivotal to our understanding of the interplay between size structure and ecosystem function.

The linear models fitted to the NSDs excluded size classes <0.5 mg because biomass for the microgram to milligram size range always fell well below that of the larger size classes (Fig. 1), making the fit of the NSD regressions to the size structure even worse if the microgram to milligram range were included. Other authors (Strayer 1986; Hanson et al. 1989; Morin and Nadon 1991) have found similar biomass deficiency for this size range. Morin and Nadon (1991) argued that periphyton likely made up most of the biomass in this size range (see Cattaneo 1987) and that NSDs would fit the microgram to milligram size range quite well if the periphyton were included in the analysis. Recently, A. Cattaneo (personal communication) has verified this hypothesis for Laurentian stream benthic communities. Since benthic invertebrate size classes <0.5 mg contribute only slightly to the biomass of the small size fraction and greatly bias the NSD parameters, and I did not analyze the size structure of the periphyton in my samples, I decided to truncate my statistical analysis at the 0.5-mg size class. Hanson et al. (1989) and Hanson (1990) also ignored these small benthic invertebrate size classes, in his case because he chose a coarser sieve mesh size and did not use a microscope to extract the animals.

Why are the Spectra Bimodal? A Hypothesis based on Life History and Seasonality

In general, larger invertebrates have longer life cycles. The animals that make up the majority of the large size fraction include large chironomids (*Chironomus*), erpobdellid leeches, lumbriculid oligochaetes, gastropods (large planorbids, physids, and lymnaeids), various odonate and trichopteran larvae, and burrowing mayfly nymphs (mostly *Hexagenia*). In most lakes within the 45–50°N latitude range, these taxa exhibit 1- or 2-yr life cycles. The animals that make up the small size fraction are mainly small chironomid larvae (e.g., *Polypedilum, Cricotopus, Endochironomus, Tanytarsus, Paratanytarsus*, and many others), small amphipods (*Hyalella*), small gastropods (*Amnicola* and *Gyraulus*), naidid oligochaetes (*Nais* and *Chaetogaster*), small tubificids, leptocerid and hydroptilid trichopterans, and small mayflies (*caenids, baetids*, and *leptophlebiids*). Most of these organisms are capable of producing two or more generations within one season. In lakes that are ice covered for nearly half the year, one should expect that overwintering is a major challenge and imposes considerable timing constraints on life cycles; thus, most species appear to have a preferred size that yields maximal overwinter survival, if not a photoperiodically timed diapause stage in their life cycle. At any rate, the strong seasonality that the animals experience in temperate-zone lakes makes it unlikely that an animal with, for example, a 9-mo life cycle, would be very successful unless it evolved some elaborate larval synchronizing mechanisms. Thus, the size range where abundance is low (8–32 mg) may correspond to the optimum size for animals with life cycles that fall in between the multivoltine group (mainly the small size fraction) and the larger animals that require 1 yr or more to complete their life cycle. This hypothesis could be tested by comparing littoral invertebrate size structures from more southerly sites, preferably with no ice cover and much less severe seasonality, with those described in this paper. To date, I know of no size spectra described for such latitudes.

It could also be hypothesized that predators (mainly fish) preferentially select prey of the size range that corresponds to the trough in the size spectrum. The fact that *Gammarus*, a favourite prey item for many fish including yellow perch (*Perca flavescens*), constitutes most of the 8–32 mg (trough) portion of the size spectra in many of these lakes lends some credence to this hypothesis. These two hypotheses are in no way mutually exclusive.

Effects of Macrophytes on Littoral Invertebrate Size Spectra

There are a number of studies that have shown the importance of aquatic macrophytes as substrates for macroinvertebrates (McGaha 1952; Cyr and Downing 1988; Rasmussen 1988a). Rasmussen (1988a) showed that the littoral zoobenthic biomass increased strongly with macrophyte abundance and that the fraction of the macroinvertebrate community found within the sediment decreased sharply with increasing macrophyte abundance. Hanson (1990) demonstrated that the littoral macroinvertebrate community size structure obtained in areas that support *Potamogeton filiformis* dominated weed-beds was consistently different from that obtained in *Chara* beds within the same lake. The invertebrate size spectra in the *Potamogeton* beds were bimodal, much like most of those seen in this study, whereas in *Chara* beds the biomass spectra were more or less uniform. In addition, Hanson(1990) found that in the *Potamogeton* beds the large size classes of invertebrates have lower biomass than the smaller size classes and the slope of the NSD was less than −1 whereas the opposite pattern was observed in the *Chara* beds. Thus the communities described by Hanson (1990) in *Potamogeton* beds are similar to those described in this study for sites with significant canopies of rooted macrophytes. None of the lakes that I studied supported *Chara* beds, and none of the size spectra that I found resembled those that Hanson (1990) found in this habitat. This suggests that the invertebrate community found in *Chara* beds has some unique and interesting features worthy of further analysis.

While Hanson's (1990) study involved only two community types, he did hypothesize that the type of vegetation present would prove to be an important overall determinant of littoral invertebrate size spectra characteristics. He also suggested that plant community variation within lakes would give rise to substantial zoobenthic size spectra variation within lakes and that within-lake variability in size spectra characteristics would be as great as between-lake variation. My analysis of the seasonal average size spectra of littoral macroinvertebrates from 53 sites in 11 lakes provided some support for the general importance of plant community structure in determining size spectra variation, although the analyses showed that total

macrophyte biomass and other limnological site variables provided stronger regression models than did macrophyte species composition. The variability in plant community biomass, trophic status, and wave exposure within Lake Memphremagog resulted in substantial within-lake variability in the littoral macroinvertebrate size spectra, and this more or less supports Hanson's (1990) prediction. The size spectra variation that I observed within this lake was, however, only about half as great as that found across my whole data set (Table 1).

Why do the Weediest Sites have the Smallest Invertebrates?

As the macrophyte biomass increases, more and more littoral macroinvertebrates live upon the plants (Rasmussen 1988a), and the community becomes more dominated by the small invertebrate size fraction. Mittelbach (1981) also found that plant-dwelling invertebrates were on average much smaller than invertebrates living in littoral sediments. The association of dense weed canopies (especially those of *M. spicatum*) with small invertebrates seems unlikely to be a direct result of the presence of the macrophytes per se, but rather, an indirect result of the invertebrates on plant stems and leaves being more exposed to size-selective predation by small fishes (Mittelbach 1988) than those hidden within the substrate. All of the sites studied here had appreciable densities of fish, in the size ranges that consume littoral invertebrates (0.2–1.4 individuals /m^2 and 2.2–46 g wet weight/m^2) (Boisclair and Leggett 1989; Pierce et al. 1990), and although their abundance (neither density nor biomass) did not explain residual variation in my regressions, they were certainly abundant enough to exert a considerable size-selective influence on the invertebrate community. Many of the large odonates, trichopterans, amphipods, leeches, and gastropods that are reported to be numerous in fishless ponds (Crowder and Cooper 1982; Morin 1984) reside primarily upon macrophytes, and these forms are generally among the first to be eliminated at high fish densities (Crowder and Cooper 1982; Mittelbach 1988; Northcote 1988). A possible explanation for the abundance of large invertebrates (and NSD slope greater than −1) in *Chara* beds (Hanson 1990) is that this type of vegetation provides an intricate meshwork that, on the one hand, is difficult for fish to penetrate but, on the other hand, does not seriously restrict water circulation or block out light. The high abundance of large invertebrates (especially leeches, odonates, and trichopterans) in dense *Chara* beds has been noted previously (Rosine 1955; Rasmussen 1979, 1982). I agree with Strayer (1991) that fish–invertebrate interactions appear to contribute in a major way to littoral zoobenthic size structure, but we lack comparable size-structure data from fishless lakes, and so I cannot presently include this factor in my models.

Regularities and Irregularities and the Interpretation of Aquatic Size Spectra

Studies of size structure in aquatic communities show certain general patterns. Normalized size spectra from planktonic communities, both marine and freshwater, can usually be described by a line with a slope close to −1, implying no biomass trend across logarithmic size classes (Sheldon et al. 1972; Sprules et al. 1983; Sprules and Munawar 1986). Simply put, the density of organisms and their size more or less linearly compensate for each other, and this is reflected in the simple allometric density–size relationship developed by Peters (1983). These regularities have been interpreted theoretically to mean that size structure reflects the flow of energy and matter from small to large organisms (Kerr 1974; Borgmann 1987); they have also been interpreted empirically to mean that abundance at any size range can be predicted using parameters, estimated from another portion of the size spectrum (Sheldon et al. 1977; Borgmann 1987; Sprules et al. 1991).

Superimposed on this broad-scale regularity are two types of irregularities: (1) departures of the density–size relationships from linearity (reflected as departures of NSD slopes from −1, or linear biomass trends across the size spectra) and (2) irregularities produced by peaks and troughs in the size structure. While this study has dealt with these two types of irregularities within the limited context of littoral zoobenthic communities over the 0.5 mg to 0.5 g size range, no broad-scale interpretation (applicable to all size classes of all aquatic communities) appears possible at this time. This study has shown that the littoral macroinvertebrate communities of the lakes studied usually have a bimodal character and that the two size modes represent independent and functionally distinct units within the benthic communities. We have also analyzed the size structure of larger (megazoobenthic) size classes, containing crayfish (0.5–16 g) and unionid clams (16–128 g), from many of the sites used in the present study. These appear to constitute two additional independent size modes that are statistically and functionally distinct and whose abundances are determined by different combinations of environmental factors (S. Lamontagne and J.B. Rasmussen, in preparation). The presence of peaks and troughs in the size spectrum that represent functionally independent size modes implies that there is no straight-forward way to (1) interpret the size spectrum theoretically as representing the flow of energy from small to large organisms and (2) make meaningful predictions of the abundance of one size fraction from size spectrum parameters estimated from another portion of the spectrum.

Strayer (1991) has pointed out a number of reasons why one should not really expect benthic size spectra to conform to the simple regularities seen in pelagic food webs, and in this light, the presence of strong peaks and troughs representing functionally independent size modes in the benthos might be considered understandable. We cannot unfortunately conclude that the theoretical and empirical utility of pelagic size structure is free of such irregularities, since strong peaks and troughs have been documented in such communities as well, most notably in the relatively complete spectra published by Sprules et al. (1991) for Lake Michigan. Although no multilake comparison using multivariate size-structure analysis is available for pelagic communities, it seems quite possible that many of the size peaks evident in such spectra will also prove to be statistically and functionally independent of one another, and if this were so, it would severely complicate their theoretical and predictive utility.

It is reasonable to hypothesize that some size fractions must be closely linked, since both bottom-up and top-down trophic interactions generally reflect interrelationships between different-sized groups of organisms (McQueen et al. 1986). Among the pelagic communities of lakes, positive relationships between phytoplankton and zooplankton (e.g., MacCauley and Kalff 1981) and between fish productivity and primary productivity (e.g., Downing et al. 1990) are well known, and, similarly, relationships between trophic factors and zoobenthic biomass have been demonstrated (Rasmussen and Kalff 1987; Rasmussen 1988a). Recently, Schallenberg and Kalff (1993) have shown that their bacteria counts from profundal mud are

quite strongly correlated with my estimates of profundal zoobenthic biomass at their study sites. In this analysis, it has, however, become clear that only the smaller size fraction of the littoral zoobenthic community exhibits a significant relationship to trophic variables and that the larger size fraction is related more to physical factors such as morphometry and wave energy than to trophic factors. Thus, to improve our understanding of size structure beyond the general density–size compensation mentioned above, we require generalized patterns among size fractions that are clearly interrelated through trophic interactions, and these must be discriminable from size-structure effects that are the product of a whole host of modifying environmental factors such as morphometry, substrate structure, and water chemistry.

Using Size Spectra Information to Estimate Community Rate Functions

The foregoing discussion has centered on the problems that certain types of statistical and structural irregularities introduce into the theoretical and empirical utility of size spectra. Regardless of whether size spectra have any broad-scale regularity or interpretability, they represent a convenient basis from which to make reasonable estimates of important rate processes of major biogeochemical importance to ecosystems (e.g., photosynthesis, respiration, uptake and excretion of P, N, or other elements, bioaccumulation and metabolism of certain contaminants) because such processes are so strongly determined by allometry (Peters 1983). Rate functions calculated by coupling allometric models with size spectra are founded on the implicit assumption that the aggregate will behave as the sum of the parts. In one of the few studies designed to test this assumption, Ahrens and Peters (1991) found that the respiration rates measured for whole plankton were more or less equal to the sum of size-fractionated rates and that the rates and allometric coefficients obtained for lake plankton communities were very similar to rates predicted from allometric models based on laboratory measurements. Although no analogous tests have been done for benthic community metabolism, this would seem to be a promising application of size structure in that it makes assumptions that are noninteractive, in complete contrast with those involved with calculating energy flow from small to large organisms, or predicting the abundance of large ones from the abundance of small ones. The use of size-structure data and models to calculate whole-system rates based on allometric models should not be sensitive to the spectral irregularities dealt with in this paper and represents a potential application of size-structural analysis to ecosystem-level modelling. Although we might not be able to interpret benthic size spectra (within the theoretical trophic framework of Kerr (1974) and Borgmann (1982, 1987), they are probably of considerable use in the prediction of ecosystem rate functions.

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Assessment of Phytoplankton Nutrient Limitation in Productive Waters: Application of Dilution Bioassays

Hunter J. Carrick,[1] Claire L. Schelske, and Frederick J. Aldridge

Department of Fisheries and Aquatic Sciences, University of Florida, 7922 NW 71st Street, Gainesville, FL 32606, USA

and Michael F. Coveney

Division of Environmental Sciences, St. Johns River Water Management District, Palatka, FL 32178, USA

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Excessive nutrient loads to aquatic systems can complicate otherwise predictable relationships between nutrient concentrations and phytoplankton biomass. We conducted six bioassays on surface phytoplankton assemblages collected from productive Lake Apopka, Florida, to measure the effect of nutrient reduction on phytoplankton growth and nutritional state. Lake water was mixed with one of three diluents to create a gradient of ambient nutrient concentrations; nitrogen (N) and phosphorus (P) limitation at each level of dilution was evaluated in a 2×2 factorial design. While the addition of N clearly increased the growth of phytoplankton in undiluted Lake Apopka water, the phytoplankton became more P limited with the reduction of particles (30–60% dilution). Regression of algal yields onto total P concentrations from our bottle experiments indicated that an 8 $\mu g \cdot L^{-1}$ change in P leads to only a 1 $\mu g \cdot L^{-1}$ change in chlorophyll yield, probably due to the high concentration of P in the lake. Because dilution influences factors in addition to ambient nutrient concentrations, results obtained with the technique must be carefully evaluated. Despite this, reduction of particles to improve water quality may, in concept, be a reasonable management scheme in lakes where a large fraction of the nutrients is particulate.

Des charges excessives en matières nutritives dans les systèmes aquatiques peuvent compliquer des relations qui seraient par ailleurs prévisibles entre les concentrations de nutrients et la biomass phytoplanctonique. Nous avons réalisé six bioessais sur des assemblages de phytoplancton superficiel prélevés dans un lac productif, le lac Apopka, en Floride, pour mesurer l'effet de la réduction des nutriants sur la croissance et l'état nutritionnel du phytoplancton. L'eau du lac a été mélangée tour à tour avec trois diluants afin de créer un gradient de concentrations ambiantes de nutriants; on a évalué la limitation de l'azote (N) et du phosphore (P) à chaque niveau de dilution à l'aide d'un plan factoriel (2×2). Tandis que l'ajout de N faisait nettement augmenter la croissance du phytoplancton dans l'eau non diluée, on observait une limitation de P avec la réduction des particules (dilution à 30–60 %). La régression de la production algale sur les concentrations de P total obtenues par nos expériences dans des bouteilles a indiqué qu'un changement de 8 $\mu g \cdot L^{-1}$ de P cause un changement de seulement 1 $\mu g \cdot L^{-1}$ dans la production de chlorophylle, ce qui est probablement dû à la forte concentration de P dans le lac. Étant donné que la dilution a un effet sur les facteurs, en plus des concentrations ambiantes de nutriants, les résultats obtenus par cette technique doivent être soigneusement évalués. Malgré cela, la réduction des particules pour améliorer la qualité de l'eau pourrait théoriquement constituer un schéma raisonnable de gestion dans les lacs où une fraction importante des nutriants se trouve sous forme particulaire.

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Although the relationship between water column nutrient concentrations (i.e., total phosphorus (TP) and total nitrogen (TN)) and phytoplankton biomass provides a useful framework for estimating the response of aquatic systems to nutrient enrichment (Dillon and Rigler 1974; Jones and Bachmann 1976), this relationship is correlative and offers limited information on underlying causal factors. Ambient nutrient concentrations are not always the best predictors of nutrient status of the phytoplankton themselves, as deficient cells can take up nutrients in excess of their immediate growth requirements (Healey 1979; Lean and Pick 1981). Several tests have been used to study the problem of nutrient limitation of phytoplankton. The most notable of these techniques are bioassays involving the enclosure of natural phytoplankton assemblages (Schelske 1984), whole-lake fertilization (Schindler and Fee 1974), and the use of physiological indicators of nutrient deficiency (Healey and Hendzel 1980; Vincent 1981a, 1981b). Despite these approaches, it can be difficult to identify the nutrients that limit phytoplankton growth in productive waters due to persistent nutrient excesses (Paerl and Bowles 1987). In these systems, alternative approaches may be required to assess nutrient limitation.

[1]Also: Department of Research, South Florida Water Management District, 3301 Gun Club Road, West Palm Beach, FL 33406, USA. Present address: Department of Biology & Romberg Tiburon Center for Environmental Studies, San Francisco State University, San Francisco, CA 94132, USA.

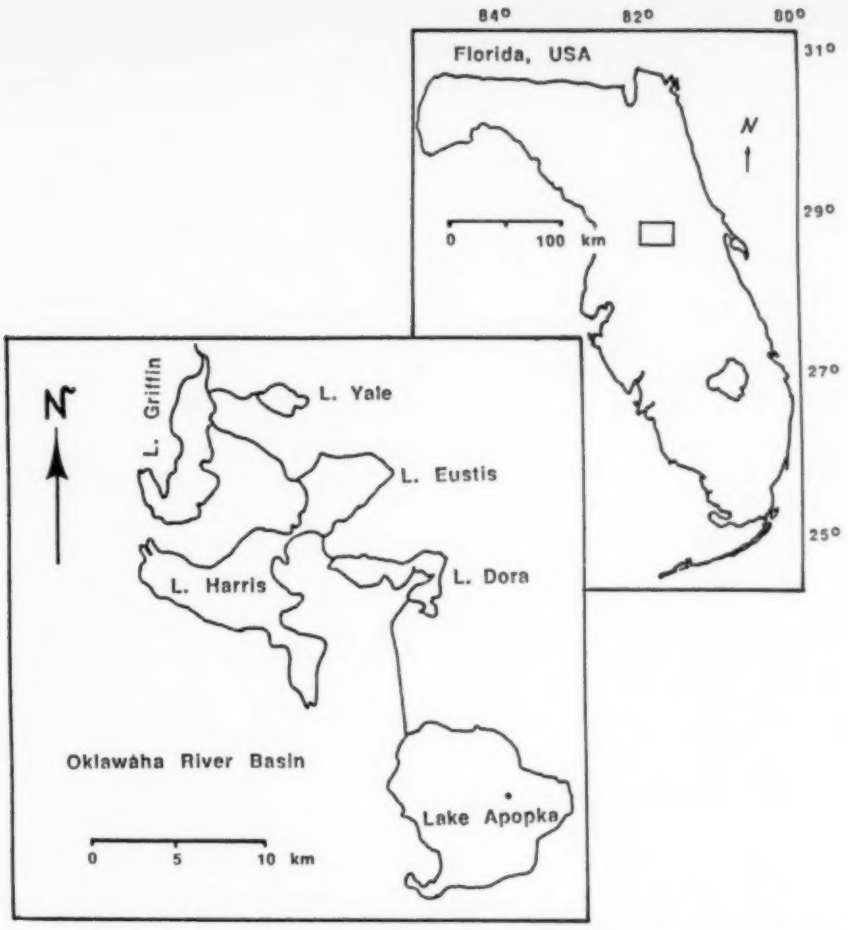

FIG. 1. Map of the location of the state of Florida, USA, with the Oklawaha River basin enlarged. The study site is indicated with a dot (after Deevey 1988; Reddy and Graetz 1991).

There is some question as to which primary factors mediate phytoplankton dynamics in tropical and subtropical lakes (Kratzer and Brezonik 1981; Osgood 1982; Canfield et al. 1989), as little research has been conducted on these systems compared with temperate systems (Lewis 1987). The evidence for nutrient limitation in subtropical lakes appears circumstantial because few controlled experiments have actually been conducted to test this hypothesis (cf. Schelske 1989). Relationships between ambient nutrient concentration (i.e., TP and TN) and phytoplankton biomass across a suite of Florida lakes support the idea that the phytoplankton biomass in these lakes is nutrient limited (Canfield 1983). However, additional work has spawned a debate as to whether phytoplankton abundance is under strict nutrient control, given the potential for self-shading at the high levels of biomass supported in some of these lakes (e.g., Agusti et al. 1990).

In this study, we evaluated nitrogen (N) and phosphorus (P) limitation of phytoplankton in productive waters utilizing an approach whereby nutrient enrichment bioassays are coupled with serial dilution of lake water (Paerl and Bowles 1987). Because dilution experiments may be prone to experimental artifacts (e.g., Li 1990; Stockner et al. 1990), the critical assumptions of the technique were also evaluated. This approach is potentially useful because it can serve as experimental evaluation of current management schemes to improve water quality in lakes, such as reduction of nutrient loads (Loehr et al. 1980) or removal of particulate material (phytoplankton and associated nutrients) from the water column of lakes via artificial wetlands (Lowe et al. 1992). The specific objectives of the study were to (1) determine if dilution alters phytoplankton N and P limitation, (2) evaluate the effect of particle reduction on phytoplankton growth yields, and (3) compare our results with the original technique formulated by Paerl and Bowles (1987).

Methods

Physical–Chemical Conditions

All experiments were conducted on surface water samples (0.3 m) collected from Lake Apopka, Florida. Lake Apopka is a

TABLE 1. Experimental conditions for nutrient dilution experiments conducted on surface water phytoplankton (0.3 m) collected on five dates in 1991. DH_2O denotes deionized water. Light exposure was 60–150 $\mu Einst \cdot m^{-2} \cdot s^{-1}$ on a 12 h light : dark cycle

Date (1991)	Diluent used	% diluent additions	Nutrient additions	Temperature (°C)
9 Jan.	DH_2O	60, 30, 0	N, P	21.5
23 Jan.	FLW	60, 30, 0	N, P, N + P	15.0
6 Feb.	FLW	60, 30, 0	N, P, N + P	19.5
26 June[a]	MIS + FLW	75, 50, 25, 0	N, P, N + P	30.0
4 Nov.	FLW	95, 90, 80, 60, 0	N, P, N + P	19.0
	MIS	95, 90, 80, 60, 0	N, P, N + P	19.0

[a]Constant phytoplankton biomass maintained among treatments.

TABLE 2. Chemical composition and final concentration of constituents in MIS used in nutrient dilution experiments based on the composition defined by Brezonik et al. (1978).

Major ion	Chemical added	Final concentration (mg ion·L^{-1})
Ca^{2+}	$CaCO_3$	35.0
Cl^-	HCl	35.0
CO_3^-	$CaCO_3$, $MgCO_3$	121.2
K^+	K_2SO_4	7.3
Mg^{2+}	$MgCO_3$	20.0
Na^+	$NaHCO_3$	15.2
SO_4^{2-}	K_2SO_4	9.0
SiO_2	Na_2SiO_3	2.1[a]

[a]Included in MIS used in 26 June experiment.

large (surface area 124 km^2), shallow (mean depth 1.7 m) lake situated at the head of the Oklawaha River chain of lakes, located 25 km northwest of Orlando, Florida (Fig. 1). This is an alkaline (pH > 9.0), clearwater lake (Brezonik et al. 1978) that is considered subtropical based on the thermal criteria outlined in Beaver et al. (1981). Soils within the Oklawaha lake basin are classified as clastic sediments relatively high in pH and mineral content, overlaying the permeable limestone of the Floridan aquifer (Brown et al. 1990). The lake is hypereutrophic relative to other lakes in Florida (Canfield 1983) as reflected by persistently high ambient nutrient and phytoplankton concentrations (Schelske et al. 1992). Currently, a restoration effort is underway to remove particle-bound nutrients from the lake via an artificial wetland (Lowe et al. 1992).

Sampling was conducted with a 5-L PVC Niskin bottle or a submersible pump at a single station in the lake (Fig. 1). Studies have shown that cells are not damaged when water is collected with a submersible pump (H.J. Carrick, unpublished data). In addition, the growth of phytoplankton in water collected by pumping was not different compared with water collected with a Niskin bottle.

On each sampling date, temperature and dissolved oxygen profiles were measured with a YSI meter (model 57). Hydrogen ion concentration was measured with a standard pH meter (Fisher Accumet model 955). Light penetration was determined with a Secchi disk (20 cm), and underwater scalar irradiance (photosynthetically active radiation (PAR)) was assessed with a LiCor LI-1000 datalogger fitted with 2π upwelling and downwelling submersible sensors. During sampling, incident irradiance (PAR) was evaluated continuously with a second LiCor LI-1000 datalogger (hourly integrated values, moles).

We separated N and P into three major chemical phases by filtration. Concentrations of dissolved inorganic forms of P (soluble reactive P (SRP)) and N (dissolved inorganic N (DIN) as NO_3-N + NO_2-N) were measured in water that had passed a 0.3-μm filter (0.3-μm Whatman 2000 EPM), while dissolved organic N (DON) and P (DOP) were determined by measuring TN and TP in this same filtered lake water fraction (<0.3 μm) and subtracting the dissolved inorganic fraction. N and P retained on the filters (>0.3 μm) was assumed to be in the particulate phase, while raw lake water was digested to determine total concentrations of N and P. In addition, storage of P in intracellular polyphosphate by the phytoplankton was estimated as hot-water-extractable P on lake water samples collected on 4 November. Subsamples were immediately filtered onto membranes (0.45-μm Gelman Supor) which were then placed in tubes and autoclaved for 60 min. This procedure hydrolyzes SRP stored in the polyphosphate bodies of the phytoplankton trapped on the filters (Fitzgerald and Nelson 1966). All nutrient concentrations were measured on a Technicon II autoanalyzer using standard colorimetric reactions (Davis and Simmons 1979).

Design of Nutrient Dilution Bioassays

Growth responses of phytoplankton to N and P were evaluated with three types of laboratory nutrient dilution bioassays (Table 1) based on bioassay techniques described by Paerl and Bowles (1987). The first type involved the serial dilution of particulate material by mixing raw lake water with either deionized water (experiment on 9 January) or filtered lake water (23 January, 6 February, and 4 November) similar to experiments designed to estimate microzooplankton grazing (Landry and Hassett 1982). In the second type of experiment, raw lake water was diluted in a stepwise manner with a major ion solution (MIS) containing only major ions dissolved in deionized water and devoid of N and P (Table 2). The addition of MIS as a diluent reduces ambient soluble nutrients present in lake water while attempting to replicate the ionic composition of ambient lake water. The third type of experiment (26 June) was designed to maintain constant phytoplankton biomass among treatments. This was achieved by mixing varying amounts of MIS, filtered lake water (FLW), and raw lake water (RLW) such that RLW constituted 25% of all treatments (constant biomass). Of the 32 bottles used in this experiment, eight contained 25% MIS as a percentage of the final volume in the flask, eight had 50% MIS added, eight had 75% MIS added, and eight contained no MIS.

Duplicate bottles from each of the dilution treatments received N and P in a factorial design, whereby two bottles were augmented with PO_4^{3-} (40 μg P·L^{-1}), two were enriched with

NO_3^- (400 μg N·L^{-1}), two received both N and P, and two did not receive nutrients and served as controls (Paerl and Bowles 1987). This design was true for all experiments except one conducted on 9 January in which N and P were added singly without a N + P treatment. Experimental bottles were incubated for 5–7 d in a walk-in growth chamber or in Percival incubators. The light (range 60–150 μEinst·m^{-2}·s^{-1}) and temperature (15–30°C) environment in the incubators was maintained at levels similar to those occurring at the average mixing depth of the lake at the time of collection (Table 1).

In all experiments, acid-washed bottles (500-mL Erlenmeyer flasks) received varying mixtures of RLW and one of three diluents to achieve a final volume of 350 mL. FLW was prepared by passing RLW through a 0.2-μm filter capsule (Gelman maxicap) under low pressure (<10 mm Hg), while deionized water used on 9 January was prepared with a reverse osmosis system.

Phytoplankton Biomass and Community Composition

In all experiments, bottles were sampled daily (every 24 h) by removing 8 mL from each flask at approximately the same time for in vivo chlorophyll *a* (Chl *a*) fluorescence determination. Because the total volume we removed from each bottle was small (<10%), we assumed that decreasing volume had little influence on the outcome of these experiments. Once collected, samples were allowed to dark-adapt for 20 min to provide a better measure of phytoplankton biomass and were then read with a Turner Designs fluorometer (Vincent 1979). Extracted Chl *a* concentrations at the start and termination of the experiments conducted in June and November were determined fluorometrically using an acetone–DMSO (50:50) extraction procedure (Shoaf and Lium 1976), although we did not use grinding.

In the experiments conducted on 26 June and 4 November, phytoplankton abundance and composition was determined using epifluorescence microscopy, due to the abundance of phototrophic picoplankton in the lake (Carrick et al. 1993). Slides were prepared within 24 h of sampling from samples preserved in 1% glutaraldehyde. Lake water subsamples (<1.0 mL) were filtered onto prestained (irgalan black) 0.2-μm pore size Nuclepore filters. Filters were mounted between a microscope slide and coverslip with immersion oil, subsequently stored at −20°C, and counted within 1 wk to minimize the fading of fluorescence (Fahnenstiel and Carrick 1992). Biomass was estimated by enumerating a total of 500–1000 individuals from two duplicate slides (<5% counting error assuming Poisson statistics) using a Nikon Research microscope (1000×) equipped for Chl *a* fluorescence (blue light 450–490 nm excitation and >515 nm emission) and determination of phycobilin proteins (green light 530–560 nm excitation and >580 nm emission). Dominant pigment fluorescence of individual picoplankton cells and intermittent observation with white light was used to assign general taxonomic (Division) position (Tsuji et al. 1986). Cell volumes were calculated for each taxon by measuring the cellular dimensions of at least 10 cells on at least two dates. These estimates were converted to carbon (C) based on Strathmann's (1967) equations for large phytoplankton (>2 μm), or the conversion factor 0.15 g C·mL^{-1} for picoplankton (Laws et al. 1984). The algal taxonomy applied conforms to that outlined by Prescott (1973) and Patrick and Reimer (1966, 1975), taking into account changes suggested by Rippka et al. (1979) and Round et al. (1990).

Data Analysis

The time-dependent change in in vivo fluorescence readings for the phytoplankton in each of the bottles was determined by calculating exponential growth rates (r) using the following:

$$r = (\ln(N_t / N_0)) / t$$

where N_t is the maximum in vivo fluorescence achieved in the experiment, N_0 is the initial in vivo fluorescence allowing 24 h for adaptation if necessary, and t is the duration of time between initial and final samplings.

Estimates of phytoplankton growth were then analyzed using a two-way analysis of variance (ANOVA), with nutrient treatments and the level of dilution considered fixed factors. When two-way ANOVA yielded significant interaction terms, one-way ANOVA was utilized where dilution was considered a blocked factor and nutrient treatments were fixed (Zar 1983). All the data met assumptions of equal variances, and Tukey's test ($p < 0.05$) was used to test for pairwise differences among the fixed factors. All statistical analyses were performed using SYSTAT (Ver. 4.0, 1991).

Evaluating Assumptions of Dilution Bioassays

Certain aspects of dilution techniques have recently come into serious question that also have bearing on other experimental protocols (Li 1986, 1990). While many of these can be readily tested, potential problems concerning passage of cells through leaky filters and contamination of diluents resulting from cell breakage require careful checks (Stockner et al. 1990). The major criticisms are four: (1) artifacts arising from containment of phytoplankton in bottles, (2) filter-induced enrichment of nutrients, (3) occurrence of cells in the diluent resulting from passage through leaky filters, and (4) contamination of the diluent after preparation. We examined a fifth assumption more specifically related to the application of the Paerl and Bowles (1987) technique, whereby the constant quantity of nutrients added to a changing level of algal biomass in the dilution treatments is thought not to alter the nutrient response of phytoplankton among the dilution treatments.

We designed experiments to investigate these criticisms, which will subsequently aid in the interpretation of results. First, to evaluate the effects of containment on Lake Apopka phytoplankton, water was collected from 0.3 m depth on 6 February 1991, mixed, and dispensed into replicate vessels of various sizes (500, 700, 1000, and 2000 mL). The volume of lake water used was allowed to vary among different-sized containers, in that we were interested in comparing our basic experimental unit (350 mL of lake water in a 500-mL flask) with that contained in a series of larger vessels. All eight vessels were incubated (as described above) at ambient light and temperature and subsampled at 0, 24, 48, 120, 140, and 160 h for extracted Chl *a*. Second, nutrient contamination of diluents was assessed by determining concentrations of N and P in the three diluents utilized in our experiments (distilled water, MIS, and FLW) using the wet chemistry methods described above. Third and fourth, the occurrence of cells in the diluents and contamination related to sampling (airborne or inadvertent inoculation of flasks) was evaluated on 4 November by incubating replicate flasks containing MIS, FLW, and each diluent with added N and P. In vivo fluorescence was measured daily and initial and final subsamples were prepared for epifluorescence microscopy to determine if

TABLE 3. Distribution of P (as SRP, DOP, and PP) and N (as DIN (NO_2-N + NO_3-N), DON, and PN) and concentrations of TP and TN for surface water collected from Lake Apopka on seven dates in 1991.

Date	SRP (%)	DOP (%)	PP (%)	TP ($\mu g \cdot L^{-1}$)	DIN (%)	DON (%)	PN (%)	TN ($\mu g \cdot L^{-1}$)
23 July	4.4	19.6	76.0	109.5	0.06	58.7	41.2	3237
24 July	3.5	10.4	87.6	137.7	0.01	49.1	50.9	3682
25 July	2.6	8.2	90.5	187.7	0.01	48.9	51.1	3682
26 July	3.5	11.9	86.6	139.3	0.04	51.8	48.1	3627
27 July	4.3	12.6	85.7	111.9	0.03	51.1	48.9	3439
16 Sept.	0.3	12.8	87.1	110.7	0.06	—	—	2968
04 Nov.	1.4	8.7	89.9	118.3	0.08	42.1	57.8	3421
Average	2.8	12.0	86.2	130.7	0.04	50.3	49.8	3436.6
SD	1.6	3.8	4.8	28.1	0.03	5.4	5.4	263.4

autotrophic cells were present and growing in the diluents.

Lastly, the effects of adding a constant nutrient spike to various standing stocks of phytoplankton (resulting from dilution) were assessed by measuring nutrient uptake in the experiments conducted on 4 November. P uptake was determined from subsamples of water from the control treatments at the start of the experiment (time 0) and from all experimental bottles after 24 h of incubation. Subsamples were immediately filtered through membranes (0.45-μm Gelman Supor) and assayed for SRP using the wet chemistry methods outlined above. Values referred to hereafter as P uptake rates (micrograms P per microgram Chl *a* per day) were derived using the following calculation:

$$\text{P uptake} = ((SRP_0 - SRP_t) / Chl) / t$$

where SRP_t is the SRP concentration at time t. SRP_0 is the initial SRP concentration, Chl is the initial Chl *a* concentration of the phytoplankton assemblage (little growth occurred in 24 h), and t is the duration of time between initial and final samplings.

Results

Nutrient Pools in Lake Apopka Surface Waters

Both TN and TP are present in high concentrations in the surface waters of Lake Apopka (Table 3). Large percentages of both nutrients occur in the particulate phase (49.7 and 85.6%, respectively). DIN and SRP occur in very low (marginally detectable) concentrations and constitute very little (<4%) of the respective TN and TP pools. Inclusion of NH_4-N concentrations measured monthly during 1991 (average 14.0, SD 9.4, n = 12, St. John's River Water Management District, Palatka, Fla., unpublished data) into our estimate shows that DIN remains a small component of N (0.5% of TN). Despite low DIN concentrations and the predominance of cyanobacteria (>80% phytoplankton C), N fixation does not appear to be an important component of the N cycle in Lake Apopka (Reddy and Graetz 1991; Aldridge et al. 1993). The remainder of N and P is present in dissolved organic fractions (51.9 and 12.6%, respectively). In addition, P stored intracellularly by the phytoplankton in polyphosphate bodies and measured as HEP constitutes a substantial fraction of particulate P (PP) in the surface waters (average 24.8%, SD 8.3, n = 3), as determined in our study and that of Newman (1991).

Nutrient Dilution Bioassays

Changes in phytoplankton growth rates among nutrient treatments were fairly consistent in all six experiments (Fig. 2 and 3). N or N + P yielded the greatest phytoplankton growth, while differences in phytoplankton growth among dilution levels were observed in five of the six experiments (two-way ANOVA). However, treatment and pairwise differences in these experiments were complex and required reanalysis, as indicated by significant interactions obtained from the two-way ANOVA (Zar 1983).

Subsequently, one-way ANOVA was used to test for differences among nutrients (dependent variable) at each level of dilution (blocked factor) in order to investigate interactions between nutrients and dilution on algal growth (Table 4). The general pattern of switching from N limitation to colimitation by both N and P among treatments was observed consistently in treatments receiving 30% or more diluent, although these differences were not always statistically significant. Viewed another way, dilution appears to relax the degree to which phytoplankton are N limited. We are confident that the in vivo fluorescence readings measured in our experiments reflect changes in algal biomass, given that in vivo fluorescence correlates well with both extracted Chl *a* ($r = 0.85$, $p < 0.001$, $n = 56$) and phytoplankton C derived from microscopic cell counts ($r = 0.68$, $p < 0.001$, $n = 36$). All three biomass estimates were determined simultaneously in the June and November experiments.

Alterations in phytoplankton composition following N + P enrichment were manifest at the termination of the three experiments from which we have data (26 June and 4 November), particularly with large dilutions of MIS (Fig. 4 and 5). In the June experiment (Fig. 4), phytoplankton biomass was constant across dilution treatments at the onset of the experiment (25% of the total mixture). By the end of this experiment, changes in the percent contribution (average percent ± 1 SD based on C) of the phytoplankton in the controls compared with initial lake water conditions (total algal C 3633 μg $C \cdot L^{-1}$, 97.3% cyanobacteria) were only observed in bottles receiving >25% MIS. Cyanobacteria (*Synechococcus* sp., *Synechocystis* sp., *Microcystis incerta* Lemm., and *Lyngbya contorta* Lemm.) were dominant in the lake (Carrick et al. 1993) and in unenriched bottles containing 0 and 25% MIS (92.4 ± 6.6 and 89.4 ± 5.3%, respectively). In the controls, chrysophytes were important (i.e., *Chrysochromulina parva* Lackey, range 7.7–75.4%) at higher levels of dilution with MIS (biomass at 0% relative to 50% dilution, 42–430 μg $C \cdot L^{-1}$). In the N + P treatments, chrysophyte biomass increased more than an order of magnitude (range 106–1193 μg $C \cdot L^{-1}$) with increasing MIS additions; these increases were greater than those realized in the controls (range in chrysophyte

biomass 17.6–44.1%). Diatoms (primarily *Synedra* sp.) only occurred in the 75% MIS treatment enriched with N + P (290 μg $C \cdot L^{-1}$, 8.9 ± 1.7% of total C).

Similar alterations in phytoplankton composition were observed in samples taken at the termination of the November experiments (Fig. 5). The percent contribution of cyanobacteria in the control treatments diluted with either FLW or MIS (81.7 ± 12.6 and 87.2 ± 8.7%, respectively) remained similar to initial lake populations (total C 3300 $\mu g \cdot L^{-1}$, 85% cyanobacteria). The addition of both N and P at large dilution factors of either FLW or MIS diluents promoted shifts in community composition, such that diatoms became more abundant (232 and 1042 μg $C \cdot L^{-1}$, respectively) and constituted a greater fraction of the total biomass (11.2 and 55.9%, respectively). This same general trend of increasing occurrence with dilution was evident for chrysophyte biomass (range 689–1033 μg $C \cdot L^{-1}$) and percent contribution (21–61%), but only when MIS was the diluent.

Algal Yield and TP Concentration

The growth yield (maximum in vivo fluorescence minus the initial fluorescence) of phytoplankton enriched with N was related to TP concentrations in five of six experiments (Table 5). TP accounted for 78–89% of the variation in algal yield, although the nature of this relationship did vary between experiments. On 9 January, no significant relationship was observed ($p < 0.20$). Maximum yields were achieved following N addition in all experiments except those on 4 November, when N + P addition prompted maximum yield (see below). The yield versus TP relationship was not relevant in the experiments on 26 June where algal biomass and TP concentrations were constant among bottles. Instead, we measured changes in algal yield with the increasing percentage of MIS added and found that algal yield (as well as diatom and chrysophyte biomass, see above) was augmented by greater additions of MIS. Lastly, fluorescence units appear to provide a good estimate of phytoplankton Chl *a* concentrations, given their strong empirical relationship with Chl *a* (see above). Also, slopes of regressions between both fluorescence units and TP and Chl *a* and TP in the June experiment gave nearly identical results (Table 5).

Relationships between algal yield and TP (or dilution) were different when plotted for individual nutrient treatments for both of the 4 November experiments. A negative relationship between maximum yield and TP was obtained for the control, P, and N + P treatments. However, yields in the N treatment increased linearly, excluding data for the 100% RLW treatment (0% dilution). These results are important for two reasons: first, the degree of N limitation of the phytoplankton appeared to be relaxed by dilution so that phytoplankton in the N-treated flasks became P limited; and second, the growth yield of phytoplankton was enhanced with increasing TP. Conversely, enrichment with both N and P promoted the greatest phytoplankton yields and these were inversely related to TP concentrations.

Major Assumptions of Nutrient Dilution Experiments

Experiments designed to evaluate the major underlying assumptions of these nutrient dilution experiments indicated that two factors we assessed did appear to complicate our results. First, MIS was intended to produce a diluent that was free of N and P and yet had an ionic composition similar to natural lake water (Tables 2 and 6). The construction of MIS was successful in the sense that this diluent had an ion balance and pH similar to natural lake water (Brezonik et al. 1978). Additions of MIS to RLW certainly reduced TN and TP concentrations, but did not diminish DIN and SRP concentrations because they were low in RLW to begin with (Table 3). Perhaps most importantly, large additions of MIS appeared to augment algal growth and changes in algal species composition beyond that which occurred

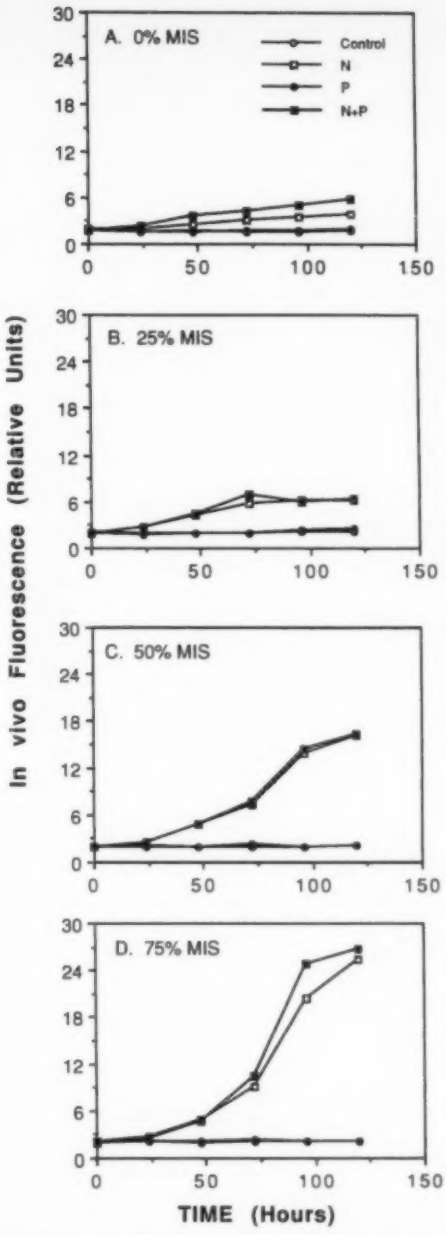

FIG. 2. Time courses of phytoplankton biomass from a nutrient dilution bioassay conducted on 26 June. Average biomass (as in vivo fluorescence) values were derived from duplicate bottles receiving one of four nutrient treatments (control, N, P, and N + P, at four dilution levels (0, 25, 50, and 75%) utilizing MIS. Phytoplankton biomass was constant among treatments at the start of the experiment.

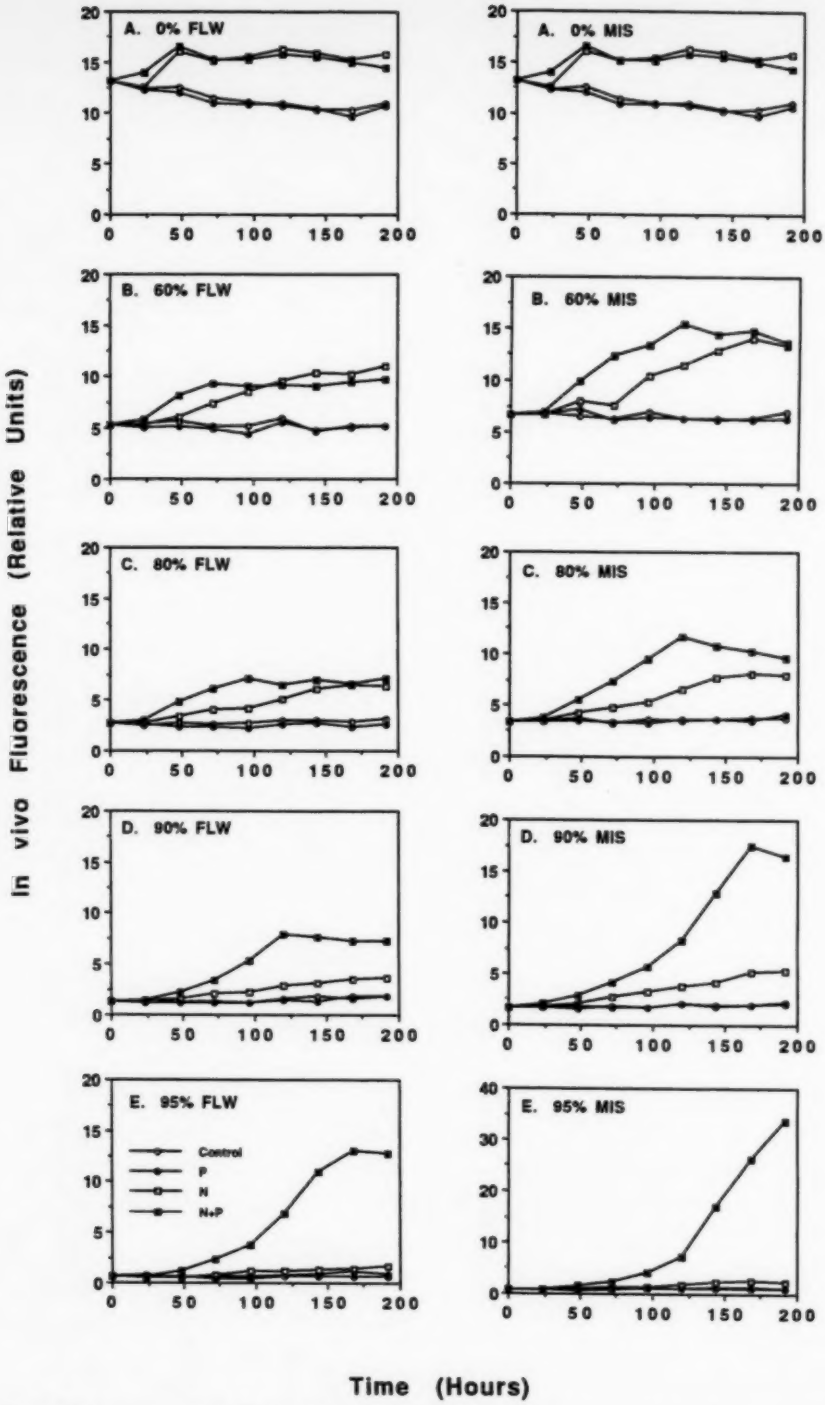

FIG. 3. Time courses of phytoplankton biomass from nutrient dilution bioassays conducted on 4 November. Average biomass (as in vivo fluorescence) values were derived from duplicate bottles receiving one of four nutrient treatments (control, P, N, and N + P) at five dilution levels (0, 60, 80, 90, and 95%). The two diluents were FLW and MIS.

TABLE 4. Results from one-way ANOVA comparing average growth rates for Lake Apopka photoplankton among nutrient treatments at each level of dilution (blocked factor). Treatments joined by underlining were not significantly different from one another when assessed with Tukey's pairwise comparisons ($p < 0.05$). ***$p < 0.00001$, **$p < 0.01$, *$p < 0.05$.

Date (diluent)	Blocked factor	*F*-stat	Tukey's pairwise comparison (least to greatest)
9 Jan.	Dilution (%)		
(DH_2O)	0	21.9**	C P N
	30	12.7*	C P N
	60	5.5	C P N
23 Jan.	Dilution (%)		
(FLW)	0	18.5***	C P N + P N
	30	91.3***	C P N + P N
	60	19.2***	C P N N + P
7 Feb.	Dilution (%)		
(FLW)	0	76.4***	C P N + P N
	30	51.7***	C P N + P N
	60	13.8**	C P N N + P
26 June	Dilution (%)		
(MIS/FLW)	0	421.6***	C P N N + P
	25	153.0***	C P N N + P
	50	261.3***	C P N + P N
	75	546.1***	C P N N + P
4 Nov.	Dilution (%)		
(FLW)	0	121.8***	C P N N + P
	60	72.9***	C P N N + P
	80	169.0***	C P N N + P
	90	2294.5***	C P N N + P
	95	40.3**	C N P N + P
4 Nov.	Dilution (%)		
(MIS)	0	121.8***	C P N N + P
	60	169.0***	C P N N + P
	80	18.0**	C P N N + P
	90	212.0***	C P N N + P
	95	202.9***	C P N N + P

following the addition of FLW (Fig. 4 and 5). At present, we do not know if inadvertent addition of elements with MIS and/or dilution of inhibitory substances in Lake Apopka water could account for the observed response.

P uptake rates varied greatly with the dilution of lake water in experiments conducted on 4 November using FLW (Table 7). After 24 h of incubation, P uptake in both P and N + P nutrient treatments were similar and increased logarithmically with increasing dilution of phytoplankton biomass. A graph of P uptake in the control and N treatments versus percent RLW showed no discernible pattern, presumably because there was little SRP available for uptake. P uptake increased linearly with the quantity of P added to our experimental bottles, once these rates were corrected for Chl *a* standing stocks (Fig. 6).

The other factors that might have confounded results did not appear to be important. None of the three diluents used in our nutrient bioassays was an additional source of N or P (Table 6). N and P concentrations were undetectable in the deionized water we used. As expected, the concentrations of DIN and SRP in the FLW produced with the filter capsule were comparable with those present in natural lake water determined with conventional filtration methods. Because the filter capsule should remove the particulate material in lake water, it is not surprising that total TN and TP concentrations in FLW expressed as a percentage of TN and TP concentrations of RLW (41.3 and 6.8%, respectively) were similar to concentrations of dissolved DON and DOP in natural lake water (Table 3).

Phototrophic cells were not present in the diluents prior to mixture with RLW, nor did algae manifest in bottles containing only FLW and MIS, even when nutrients were added to promote their growth. Daily in vivo fluorescence readings of these bottles and inspection of subsamples with epifluorescence microscopy showed that phototrophic cells were not detectable in the diluents as found in some studies (Li 1990). This is not to say that these diluents did not support the growth of other planktonic components such as heterotrophic bacteria and protozoans that would not have been easily detected due to their lack of autofluorescence. While stains were not used to confirm the presence or absence of heterotrophic organisms, we believe their presence was unlikely because no trace of them was observed under the bright background fluorescence of green light excitation.

Lastly, containment artifacts attributable to the use of 500-mL bottles in the experiments were probably small. Minor differences were observed in the growth (-0.01 to $-0.04 \cdot d^{-1}$) of phytoplankton contained in bottles of varying size (500 to 2000 mL) relative to the growth of phytoplankton following nutrient addition in our dilution experiments.

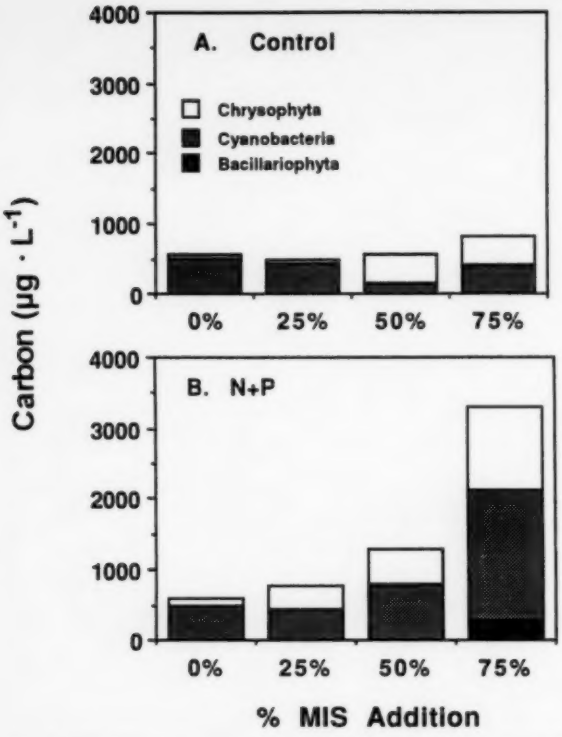

FIG. 4. Phytoplankton community composition and biomass (as C) for a nutrient dilution bioassay conducted on 26 June. Average biomass values were derived from microscopic cell counts taken from duplicate bottles receiving one of two nutrient treatments (control and N + P) at four dilution levels (0, 25, 50, and 75%) where MIS was the diluent.

Discussion

Nutrient Limitation in Productive Waters

The addition of N in our experiments clearly increased the growth of phytoplankton in undiluted Lake Apopka water in the laboratory; however, reduction of particles in lake water altered the response of the phytoplankton to nutrients. This was particularly true at dilution factors of 30% or greater, when phytoplankton growth appeared to become more limited by P. This was a consistent feature in nearly all of our experiments independent of the diluent utilized, although additional growth stimulation was achieved in treatments receiving the greatest dilution with major ionic solution, MIS. High proportions of N and P present in particulate matter rather than in soluble reactive forms made it possible to remove nutrients with FLW additions, while mixtures of N and P-deficient MIS may be required to dilute abundant dissolved nutrients even though MIS may also contain unwanted substances. The later situation was the case in the Neuse River, North Carolina, where dissolved inorganic nutrient concentrations were very high (approximately 200 $\mu g \cdot L^{-1}$) and readily reduced with additions of MIS (Paerl and Bowles 1987). Following another approach, Dodds and Randel (1992) used chemical treatments (alum, zeolite) to remove high concentrations of dissolved inorganic nutrients from lake water.

P limitation of freshwater phytoplankton has been demonstrated in a variety of environments, suggesting that N limitation is less common. N limitation may be more prevalent in marine systems (Hecky and Kilham 1988; Vitousek and Howarth 1991). However, recent work in subtropical and tropical lakes indicates that N limitation of phytoplankton may be more frequent than previously thought, particularly in productive lakes like Lake Apopka (Henry et al. 1985; Agusti et al. 1990). Moreover, reanalysis of experiments on temperate lakes suggests that N limitation and N + P limitation are more prevalent than P limitation (Elser et al. 1990), and N utilization can be an indicator of the severity of eutrophication (Schelske 1975). Our results support the hypothesis that N limitation is a consistent feature in some freshwater systems, particularly eutrophic to hypereutrophic lakes.

The nutrient status of productive freshwater lakes, like Lake Apopka, may be similar to some estuarine habitats. Like many estuaries (e.g., Howarth 1988), Lake Apopka is consistently N limited as determined from 20 bioassay experiments conducted over an 18-mo period (Aldridge et al. 1993). DIN (including both NO_2-N + NO_3-N and NH_4-N) to SRP molar ratios are low (average ratio 11:1, $n = 7$) relative to the Redfield (1958) N:P molar ratio of 16, and large concentrations of DON and DOP are common (see Table 3). Also, nutrient loads to the lake are low in N relative to P compared with the Redfield ratio (4:1, Lowe et al. 1989). These patterns are commonly observed in estuarine environments (Boynton et al. 1980).

Predicted Changes in Algal Yield with P Loading

Relationships between algal biomass and TP loads have long been used to assess the nature of lake eutrophication (Vollenweider 1968). Reductions in P loading should result in lower algal biomass provided the algae are growth limited by P at some point (Tilman 1982). However, this relationship may be complicated. For example, luxury P uptake and storage by the phytoplankton commonly occurs when P loads are excessive (e.g., Lean and Pick 1981; Schelske and Sicko-Goad 1990), at which point the phytoplankton may become growth limited by other substances, thus decoupling the relationship between P loads and algal biomass.

This decoupling appears to be the case in Lake Apopka, where P loads are high relative to N (average TP > 100 $\mu g \cdot L^{-1}$, see Table 3). A large portion of the TP is stored within phytoplankton cells as readily soluble polyphosphates (24% of PP, $n = 3$) and very little P is present in a soluble reactive form (average SRP 2.8%, $n = 7$). Consequently, the phytoplankton in Lake Apopka are not currently growth limited by P; however, they do become more P limited with increasing dilution (see Fig. 6).

The slope of the regression (k) between TP and Chl *a* yields derived from our experiments has predictive value in determining the degree of P overloading in this and other ecosystems. Our experiments were designed to determine the change in algal yield (in vivo fluorescence units (FU); dependent variable) expected per unit increase in TP (independent variable), as expressed by the slope of this regression. The average slope from our N addition experiments was 0.129 $FU \cdot (TP \cdot L^{-1})^{-1}$ (coefficient of variation 30.2%), given the range of concentrations tested. This indicates that currently in Lake Apopka, a 1 $\mu g \cdot L^{-1}$ change in Chl *a* concentration would require a reduction in TP of approximately 8 $\mu g \cdot L^{-1}$.

The relationship between TP and summer Chl *a* concentrations for some temperate lakes yields a slope of nearly 1 (Dillon and Rigler 1974; Jones and Bachmann 1976); however, a slope <1 in

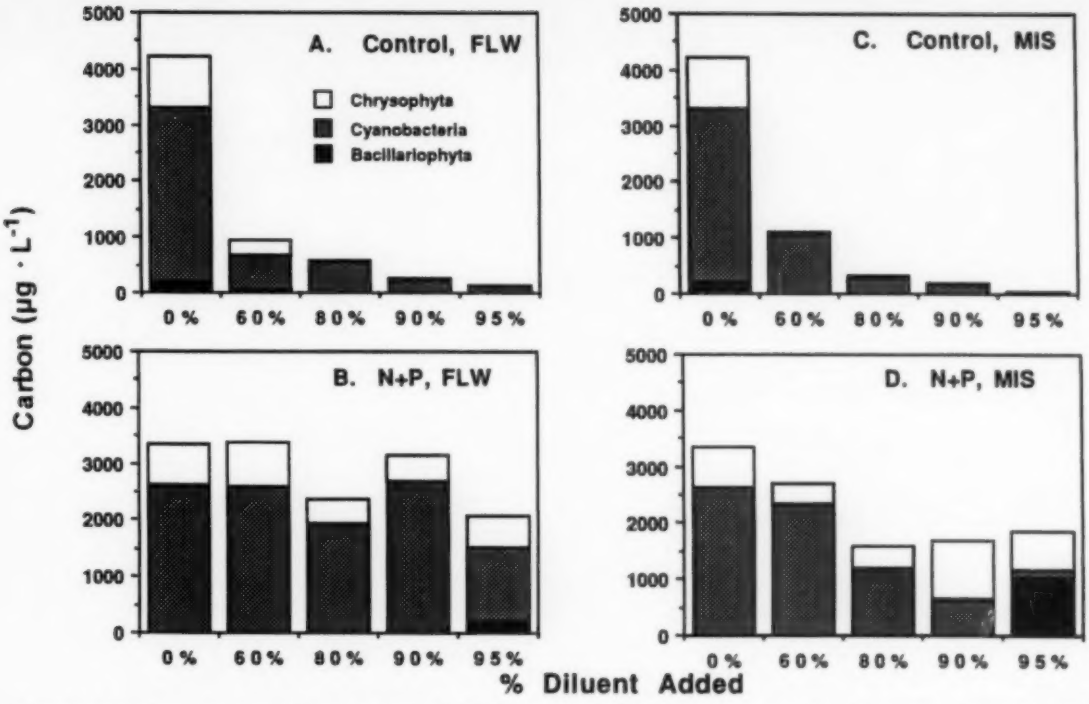

FIG. 5. Phytoplankton community composition and biomass (as C) for nutrient dilution bioassays conducted on 4 November. Average biomass values were derived from microscopic cell counts taken from duplicate bottles receiving one of two nutrient treatments (control and N + P) at five dilution levels (0, 60, 80, 90, and 95%). The two diluents were FLW and MIS.

TABLE 5. Summary of linear regression analyses assessing the relationship between the maximum yield measured with in vivo fluorescence (relative units from the N addition treatment) and TP ($\mu g \cdot L^{-1}$) for five nutrient dilution experiments (variable biomass). In addition, regression analyses evaluating the relationship between maximum algal yield and percent MIS added were conducted on one date (constant biomass). All experiments were performed on surface phytoplankton communities collected from Lake Apopka.

Diluent	Date	*y*-intercept	Slope	*n*	r^2	Prob
		Variable biomass				
DH_2O	9 Jan	1.20	0.087	6	0.42	0.200
FLW	23 Jan.	0.85	0.131	6	0.86	0.005
	6 Feb.	2.49	0.182	5	0.78	0.050
	4 Nov.	0.42	0.097	8	0.79	0.002
MIS	4 Nov.	1.33	0.150	8	0.86	0.001
		Constant biomass				
MIS	26 June	0.98	0.290	8	0.89	0.001
	26 June[a]	14.37	0.288	8	0.83	0.005

[a]Relationship based on extracted Chl *a* ($\mu g \cdot L^{-1}$).

the TP – Chl *a* relationship among lakes is not uncommon (Forsberg and Ryding 1980; Canfield 1983). Both of the later studies observed nonlinearity in the relationship at high TP concentrations (>100 $\mu g \cdot L^{-1}$) that accounted for lower slopes, while the studies by Dillon and Rigler (1974) and Jones and Bachmann (1976) had fewer samples from lakes of greater trophic state. If TP concentrations in Lake Apopka were reduced by 30–60% as suggested by our experiments (range 50–90 $\mu g \cdot L^{-1}$), correspondingly, Lake Apopka would fall onto the linear portion of this empirical TP – Chl *a* relationship.

Our data offer experimental evidence to suggest that N limitation can explain some of the discrepancy in TP – Chl *a* relationship for Lake Apopka. Such departures or nonlinearity are cited as evidence that phytoplankton biomass is limited by substances other than P, most likely N or light (Canfield 1983). The slope of the TP – Chl *a* relationships derived from our experiments ($k = 0.129$ µg Chl·µg TP^{-1}) was only comparable with the empirical data collected by Canfield from 165 Florida lakes ($k = 1.80$ µg Chl·µg TP^{-1}) when lake water was enriched with N. The studies of Aldridge et al. (1993) also indicate that

TABLE 6. Average (SD) concentrations of P (as SRP and TP) and N (DIN as NO_2-N + NO_3-N and TN) for three diluents used in nutrient dilution experiments. These values are compared with values for RLW collected (0.3 m) on 4 November from Lake Apopka. Estimates were derived from duplicate water samples.

Diluent	SRP ($\mu g \cdot L^{-1}$)	TP ($\mu g \cdot L^{-1}$)	DIN ($\mu g \cdot L^{-1}$)	TN ($\mu g \cdot L^{-1}$)
DH_2O	0.0	0.0	0.0	0
FLW	1.8 (0.7)	7.2 (0.0)	0.6 (0.1)	1295 (29.7)
MIS	1.4 (0.2)	5.9 (0.6)	0.9 (0.9)	23 (26.9)
Lake	2.0 (0.4)	105.8 (3.4)	0.8 (0.7)	3136 (128.7)

TABLE 7. Average P uptake rates (μg P·μg $Chl^{-1} \cdot d^{-1}$) by Lake Apopka phytoplankton exposed to four nutrient treatments and diluted with various quantities of FLW. Uptake estimates were derived from duplicate water bottles containing lake water collected on 4 November and incubated for 24 h. Extracted Chl *a* values were measured at the outset of the experiment.

% RLW	Chl *a* ($\mu g \cdot L^{-1}$)	Nutrient treatment			
		Control	N	P	N + P
5	4.44	0.04	0.04	7.16	7.08
10	8.49	0.10	0.11	4.08	4.02
20	16.81	0.02	0.01	2.10	2.10
40	31.42	0.01	0.02	1.13	1.13
100	91.12	0.01	−0.01	0.35	0.37

phytoplankton growth in Lake Apopka is N limited. Further algal growth following additions of MIS in our experiments suggests that other elements (in addition to N) enhance algal growth or dilute some inhibitory substance that retards growth.

This is not to discount the potential importance of light in turbid systems. Light can compensate the response of phytoplankton to nutrients, particularly at levels below light saturation (Rhee and Gotham 1981; Fahnenstiel et al. 1984). Because a significant portion of the shallow water column (mean depth 1.7 m) in lake Apopka received <1% surface irradiance (approximately 33%) due to the turbid nature of the water, it is likely that phytoplankton yield is diminished to some extent by low light. These dynamics could also account for disparities in the nutrient – Chl *a* relationship in shallow, turbid systems like Lake Apopka.

Factors Influencing Nutrient Dilution Bioassays

Several factors can influence the results derived from nutrient dilution bioassays, in addition to those evaluated when the method was first presented (Paerl and Bowles 1987). We found that factors such as bottle containment, N and P contamination of the diluents, and the occurrence and growth of cells in the prepared particle-free diluents did not significantly influence our results, although these factors can be important (Li 1986, 1990). Nonetheless, we feel such checks are important when using this bioassay procedure, especially because factors like containment can affect specific planktonic species (Carrick et al. 1992) or are manifest in a manner that is temporally variable (Fahnenstiel and Scavia 1987).

Several experimental artifacts appeared to influence our results and thus temper our interpretation of them. The phytoplankton response to nutrient enrichment was not always similar among the three diluents. MIS promoted higher phytoplankton yields and changes in community composition compared with the FLW diluent. This observation was confirmed in the constant biomass experiment (26 June), where algal yield was measured as a function of increasing percent MIS (see Table 5).

The stimulatory effects associated with large additions of MIS might be explained in one of several ways. Important trace elements or other micronutrients can be present in manufactured chemicals and might have been present in the analytical, reagent-grade chemicals from which we constructed MIS. If so, we inadvertently added such substances to our experimental bottles, particularly elements like Si which have been identified as common contaminants (e.g., Carrick and Lowe 1988). The presence of Si in MIS might account for the additional growth of diatoms and chrysophytes we observed. Removal of natural chelates (present as dissolved or particulate material) with additions of MIS might have increased the availability of other micronutrients that otherwise would have been unavailable. While we added more MIS to some treatments compared with the work of Paerl and Bowles (1987), we did observe changes in algal growth and composition at relatively low additions as well (25–95%). At any rate, alterations in the supply rate of nutrients or trace elements, resulting from dilution, could influence algal growth and composition (e.g., Kilham and Kilham 1984).

Inhibitory substances in Lake Apopka water might have been diluted with the addition of MIS and not FLW. Given that phytoplankton biomass in Lake Apopka is dominated by cyanobacteria (Carrick et al. 1993), the lake may behave like some other systems where alleopaths produced by the cyanobacteria can suppress other phytoplankton groups, particularly diatoms (Keating 1978). The dramatic shift from cyanobacteria to diatom dominance following dilution with MIS supports this notion because additions of MIS would dilute any inhibitory chemicals present in Lake Apopka water. Regardless, confounding factors such as those reviewed above need to be considered in future studies.

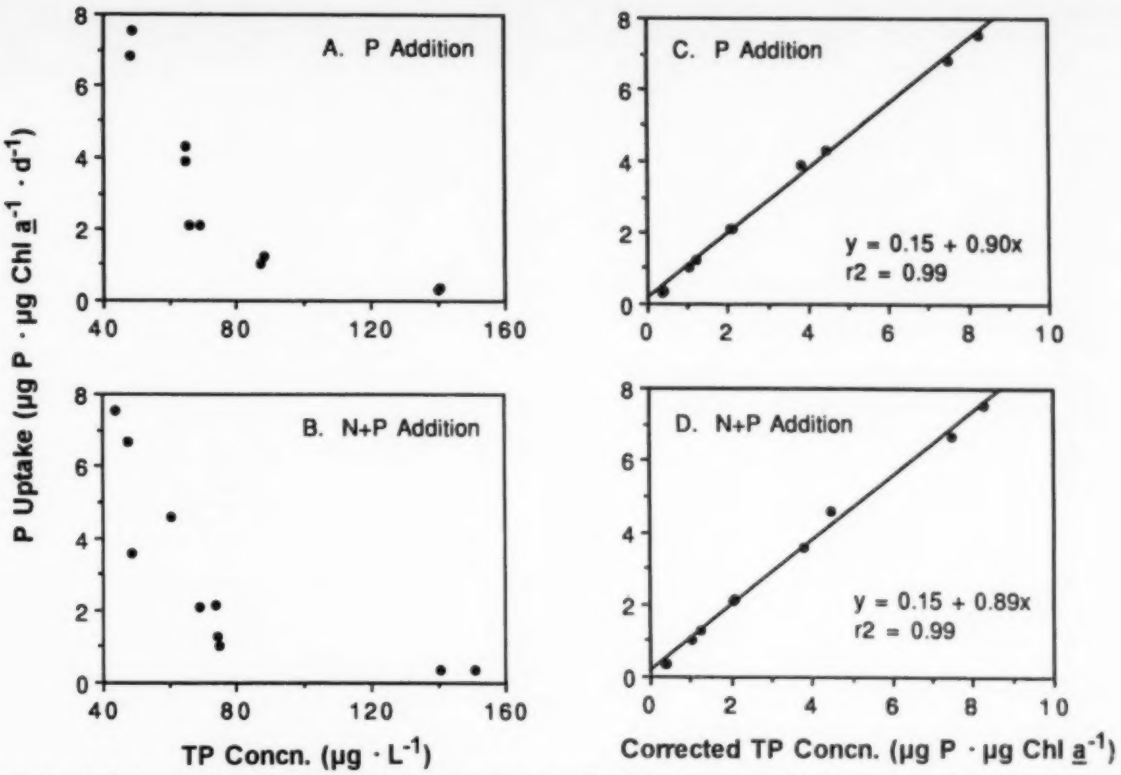
A. P Addition
B. N+P Addition
C. P Addition
y = 0.15 + 0.90x
r2 = 0.99
D. N+P Addition
y = 0.15 + 0.89x
r2 = 0.99
P Uptake (µg P · µg Chl a-1 · d-1)
TP Concn. (µg · L-1)
Corrected TP Concn. (µg P · µg Chl a-1)

FIG. 6. (A and B) Average P uptake rates versus TP concentrations and (C and D) linear relationship between P uptake rates and corrected TP concentrations (relativized to Chl *a*). Lake Apopka phytoplankton were obtained from nutrient dilution bioassays conducted on 4 November. Uptake estimates were derived from duplicate water bottles receiving one of two nutrient treatments (P and N + P) at five dilution levels (0, 60, 80, 90, 95%) with FLW.

The question of why phytoplankton become more P limited with dilution (or why the degree of N limitation is relaxed with dilution) is difficult to answer. Because we added the same dose of nutrients to experimental bottles, the concentration of added nutrients per unit phytoplankton biomass changed along our dilution series, although the N:P ratio did not. This meant that the quantity of added soluble inorganic N or P encountered by the phytoplankton at 95% FLW dilution was approximately 20-fold higher than that available to undiluted phytoplankton. The pattern of increasing P uptake rates by phytoplankton with dilution appears to be analogous to experiments designed to determine uptake kinetics by exposing phytoplankton to increasing concentrations of nutrients (e.g., Dugdale 1967). Instead of adding greater concentrations of nutrients, we have reduced the biomass of phytoplankton; hence, a characteristic hyperbolic relationship exists between the corrected P concentration added to our bottles and phytoplankton growth (Monod 1950).

Associated changes in algal yield expressed as a function of TP can be complex because manipulated TP concentrations covary with other physical and biological parameters. For example, the design of our experiments is similar to that utilized to estimate microzooplankton grazing (Landry and Hassett 1982). In Landry and Hassett's experiments, increasing lake water dilution relieves grazing pressure, such that regressing the growth rate of phytoplankton prey (dependent variable) onto the corresponding dilution treatment (independent variable) yields estimates of microzooplankton grazing loss (slope) and the intrinsic growth rate of the prey (*y*-intercept). Despite this, interference due to microzooplankton grazing did not appear to be a significant factor in our experiments. Algal growth in control and P treatments was influenced only to a limited extent by microzooplankton grazing, whereby derived loss rates were low (average slope ± SD: $-0.0011 \pm 0.0001 \cdot d^{-1}$) relative to growth (average *y*-intercept ± SD; $0.072 \pm 0.007 \cdot d^{-1}$). More importantly, estimates of algal growth in the N-enriched treatments (range $0.1–0.4 \cdot d^{-1}$) were greater by more than two orders of magnitude than losses attributable to microzooplankton grazing. While errors in the algal yield versus TP relationship were not attributable to microzooplankton here, the importance of this factor cannot be neglected, particularly because the relative importance of microzooplankton grazing varies with changing food web structure (e.g., Pace and Funke 1991).

Application of Dilution Bioassays

The application of nutrient dilution experiments to the study of phytoplankton nutrient limitation in eutrophic waters, like Lake Apopka, is important for several reasons. First, the large magnitude of nutrients incorporated into algal biomass can be indicative of the degree of high nutrient loading that exists. Such a condition is difficult to evaluate using classical approaches which assess relationships between phytoplankton biomass and

P loading (e.g., Jones and Bachmann 1976). Second, assessment of phytoplankton nutrient status along a series of lake water dilutions can be utilized for predictive purposes, utilizing FLW to dilute particulate nutrients or MIS to reduce dissolved nutrients. Third, this type of experimentation can offer independent validation for target nutrient reduction plans required to alleviate the cultural eutrophication of marine and freshwaters. A wetland was constructed adjacent to Lake Apopka in an effort to reduce annual TP loads by 30% (Lowe et al. 1992), while target management plans for eutrophic Lake Okeechobee, Florida, are currently set at 40% reduction in P loading (Federico et al. 1981) by employing best management practices to the agriculture surrounding the lake. Nutrient dilution experiments could be applied to evaluate such nutrient reduction practices, with recognition of the methodological constraints inherent to these experiments. Because Lake Apopka has high P loads and much of these are stored as intracellular polyphosphate by the phytoplankton (24% of PP), reduction of lake water particles is at least conceptually a reasonable management approach.

We evaluated some inherent features of nutrient dilution experiments. Some of these factors influenced our results and need to be considered prior to the application of this method. Such factors include (1) potential biases related to the diluents used, (2) the amount of nutrient added in the bioassays relative to variation in phytoplankton biomass imposed by dilution, and (3) impact of microzooplankton grazing losses.

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Importance of Runoff and Winter Anoxia to the P and N Dynamics of a Beaver Pond

Kevin J. Devito[1]

Watershed Ecosystems Program, Trent University, P.O. Box 4800, Peterborough, ON K9J 7B8, Canada

and Peter J. Dillon

Dorset Research Centre, Ontario Ministry of Environment and Energy, Bellwood Acres Road, P.O. Box 39, Dorset, ON P0A 1E0, Canada

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Annual retentions of total phosphorus (TP) (–11%) and total nitrogen (TN) (–5%) of a headwater beaver pond in central Ontario were low during 1987–88. Annually, inputs exceeded outputs of total reactive P (71%) and NO_3-N (35%), and outputs exceeded inputs of total unreactive P (–33%) and total organic N (–26%), and inputs approximated outputs of NH_4-N (–8%). Seasonal trends in P and N retention were inversely correlated with runoff. Monthly retention was weakly positively related to average water temperature and redox potential (ORP). Positive monthly retention coincided with low runoff and high biotic assimilation during the growing season. Winter ice cover was associated with undetectable dissolved oxygen and low ORP and increased levels of P and N, particularly NH_4-N. High levels of P and N in the surface water during winter were coupled with increased runoff and potentially low biotic assimilation resulting in a net release of TP and TN. Large flow-through of waterborne inputs and flushing of regenerated P and N occurred during peak snowmelt resulting in low annual retention. Initial accumulation of flooded forest material and input of organic matter by beaver may be very important to P and N dynamics, representing a long-term source of nutrients to the pond water and outflow.

Les rétentions annuelles de phosphore total (PT) (–11 %) et d'azote total (NT) (–5 %) dans un étang à castors situé en altitude dans le centre de l'Ontario étaient faibles en 1987–1988. Chaque année, les apports dépassaient les sorties en ce qui concerne P réactif total (71 %) et NO_3-N (35 %), les sorties dépassaient les apports de P non réactif total (–33 %) et de N organique total (–26 %) et les apports étaient à peu près égaux aux sorties pour NH_4-N (–8 %). Les tendances saisonnières de la rétention de P et N étaient inversement corrélées au ruissellement. La rétention mensuelle était faiblement liée de façon positive à la température moyenne de l'eau et au potentiel d'oxydoréduction (POR). La rétention mensuelle moyenne coïncidait avec un faible ruissellement et une forte assimilation biotique au cours de la saison de croissance. La couverture de glace hivernale était associée à une concentration non détectable d'oxygène dissous et à un faible POR, ainsi qu'à une augmentation des concentrations de P et de N, particulièrement NH_4-N. Les concentrations élevées de P et de N dans l'eau de surface en hiver étaient couplées à un accroissement du ruissellement et à une assimilation biotique potentiellement faible débouchant sur une libération nette de PT et de NT. Pendant la période de pointe de la fonte des neiges, on observait une forte circulation des apports dus à l'eau et une chasse de P et N régénérés, ce qui expliquait la faible rétention annuelle. L'accumulation initiale de matériaux forestiers submergés et l'apport de matière organique par les castors peuvent être très importants pour la dynamique de P et N, ce qui représenterait une source à long terme de matières nutritives pour l'eau de l'étang et son exutoire.

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Beaver (*Castor canadensis*) dams and ponds are ubiquitous throughout central Ontario and much of North America. They often are positioned such that much of the runoff from a catchment must pass through them as it moves from terrestrial to downstream ecosystems. As a result, beaver activity can greatly influence the export and transformation of essential nutrients, such as phosphorus and nitrogen, from headwater catchments to downstream surface waters (Naiman et al. 1987; Dillon et al. 1991).

Few data related to P and N dynamics in beaver ponds exist despite their possible influence and relative abundance. The available information suggests that beaver ponds exhibit variable nutrient retention efficiencies. Devito et al. (1989) reported low retention of waterborne total phosphorus (TP) and total nitrogen (TN) in two beaver ponds on the Precambrian Shield of central Ontario. Devito et al. (1989) measured a net retention of inorganic N and net release of organic N, but analysis of the forms of P has not been done. Dodds and Castenholz (1988) reported a large flow-through of N in a beaver-inhabited spring pond in Oregon. Sequestering of P and N in deposited sediments has been reported in mountain areas of Wyoming (Maret et al. 1987) and the Precambrian Shield area of Quebec (Naiman and Melillo 1984).

[1]Present address: Department of Geography, York University, North York, ON M3J 1P3, Canada

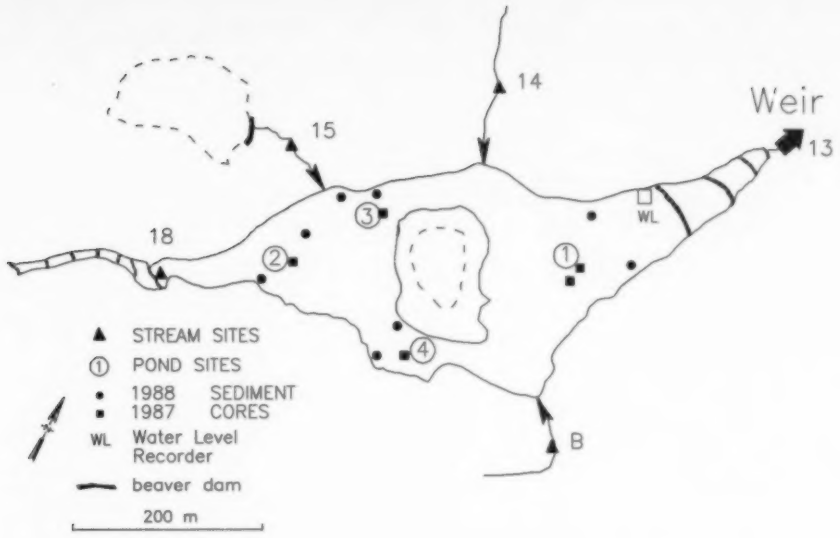

FIG. 1. Beaver pond with approximate locations of pond (Nos. 1–4) and stream (Nos. 13, 14, 15 and B) sampling sites, coring locations, and water level recorder.

Detailed studies examining the processes influencing P and N transport and mobilization in beaver ponds are lacking. Both hydrologic and biochemical process influence nutrient export and cycling in streams and wetlands. Water acts as a vehicle for export, and the losses of dissolved and particulate substances have been related to the magnitude of runoff and water retention time (e.g., Meyer et al. 1981; Hill 1988). The hydrologic mobility of P and N may be controlled by processes in the sediments and surface waters which limit or enhance the transfer of nutrients to the hydrologic component. The dominance of anoxic processes in regenerating nutrients and introducing them into the water has been observed in beaver ponds and lakes (Dahm et al. 1987; Carignan and Lean 1991), but they have not been directly related to seasonal or annual retention of P and N within a stream reach. Knowledge of the interaction of hydrology and anoxic processes in beaver ponds and how these vary seasonally is necessary to develop generalizations about the role of beaver ponds in the nutrient dynamics in headwater catchments.

Seasonal variations in P and N retention in a number of wetlands on the Precambrian Shield was described by Devito et al. (1989). Annual retention of TP and TN within the wetlands was the difference between positive retention during the "ice-free" season and negative retention during the winter and spring. The seasonal budgets of other beaver ponds on the Precambrian Shield have not been reported. Seasonal dynamics have been reported in other types of wetlands, with export of nutrients occurring only during certain times of the year (Atchue et al. 1983; Elder 1985). This suggests that nutrient flux and transformations may be controlled by seasonal variations in hydrology, anoxia, and/or temperature related biotic assimilation.

This study is part of an effort to evaluate the influence of beaver impoundments on nutrient export from forested catchments on the southern Precambrian Shield. We examine the interaction of hydrology, water anoxia, temperature, and nutrient concentrations within a beaver pond in conjunction with a detailed mass balance to test the hypotheses that (1) the seasonal retention, and thus annual retention, is a function of hydrology and (2) the seasonal retention is a function of pond water anoxia associated with ice cover. Water temperature and P and N chemistry were used to infer the influence of biotic assimilation on P and N retention within the pond.

Study Area

The beaver pond is located on the course of a small stream flowing into Harp Lake (45°23′N, 79°08′W), draining one of six subcatchments (Harp Lake subcatchment 4) of the lake. The lake is situated near the southern border of the Precambrian Shield, in central Ontario, Canada. The mean annual January and July air temperatures in the study area are −11.0 and 17.7°C, respectively, and water bodies are generally ice-covered from about the beginning of December to the middle of April. The area receives 900–1100 mm precipitation/yr with 240–300 mm falling as snow. Each month during the period of snow and ice-cover, some precipitation falls as rain. The long-term annual runoff is 400–600 mm/yr. A more detailed description of the climate and physiography, geology, and geochemistry of the area and at Harp 4 subcatchment has been reported by McDonnell and Taylor (1987), Devito et al. (1989), and Dillon et al. (1991).

The beaver pond is on a third-order stream about 500 m upstream from Harp Lake. The pond (3.8 ha) has a catchment area of 61.5 ha. Information from air photos shows the pond being formed between 1960 and 1969. It is a shallow (1.2 m average depth), steep-sided, dystrophic pond with floating mats of *Sphagnum* spp. and Labrador tea (*Ledum groenlandicum*) along the shore. Dense growth of macrophytes (primarily *Potamogeton* spp.) was initially observed during late winter under the ice and remained until July. Beavers flooded a low-lying forest, and numerous dead tree snags still stand throughout much of the pond. A small valley bog (5 m depth) was also flooded, and a small ring (0.59 ha) of floating *Sphagnum*, *L. groenlandicum*,

and eastern larch (*Larix laricina*) remains in the centre of the pond (Fig. 1). Beaver activity is sporadic as trappers periodically remove the animals; however, fresh cuttings were observed and new material was added to the dams throughout the 1987–88 study period, suggesting that at least one beaver was present.

There are several ephemeral channelized inflows into the pond. Streams 15, 14, and B drain moderately sloped uplands of primarily deciduous forests; the former contains a small beaver pond (Fig. 1). The main inflow, stream 18, drains a substantial portion of the catchment (39.2 ha) which itself contains a large conifer swamp and a beaver pond. Unchannelized inputs derived from the area adjacent to the pond (9.7 ha) drain moderate-grade uplands of deciduous forests with small stands of conifers. The depth of overburden surrounding the beaver pond ranges from 0 to 2 m.

Methods

Precipitation and air temperature data were obtained from a meteorological station located within 1 km of the pond. Rainfall and snow depths were measured using Belfort rainfall gauges. Stream discharge at the mouth of Harp Lake subcatchment 4 and the beaver pond outflow, stream 13, was continuously monitored with F-type Leupold–Stevens recorders at 90° V-notched weirs. From March 1987 to June 1988, instantaneous discharges of the inflow streams to the beaver pond were measured using Ott current meters or a graduated bucket at a convenient location along the stream channel. Measurements were made at least once a week, but more frequently (often twice daily) during peak flow. Mean daily discharge data were calculated by linear integration of instantaneous discharge measurements (Scheider et al. 1979). Discharge at stream 18 was estimated by a regression relationship between instantaneous discharge at stream 18 and that at outflow stream 13. Runoff from ungauged areas adjacent to the pond was estimated from the unit area runoff at stream 13 during the study period. Prior to 1987–88 the runoff at the outflow and inflow streams to the pond was estimated by prorating unit area runoff for the entire Harp Lake subcatchment 4 as described by Devito et al. (1989).

Water level in the pond was continuously monitored during the ice-free period of 1987 and 1988 with a Leupold F-recorder (Fig. 1). Staff gauge readings were recorded on a daily to weekly basis during the other periods.

Bulk (wet and dry) deposition was collected once a week from Teflon-coated bulk collectors. Prior to 1987–88, study stream samples were collected one to three times per week depending on the time of year. During 1987–88, stream and pond water samples were collected one to seven times a week depending on runoff. Water samples were collected in prerinsed Teflon or glass bottles and filtered through 80-µm Nitex mesh in the field. Surface water of the beaver pond was sampled at two depths, 0.3–0.5 and 0.9–1.1 m below the water surface, at each of the four sites (Fig. 1). During the winter, sampling was carried out through holes cut in the ice with AVS pipe frozen in place to prevent surface rain and meltwater draining into the underlying water column.

TP, total Kjeldahl nitrogen (TKN), ammonium-nitrogen (NH_4-N), nitrate/nitrite-nitrogen (NO_3-N), and chloride (Cl) were determined as outlined in Ontario Ministry of the Environment (1981). Total reactive phosphorous (TRP) was determined as outlined by Stainton et al. (1977). The platinum/calomel electrode used for oxidative redox potential (ORP) measurements was standardized with Zobell solution (Zobell 1946). The calomel electrode potential (EM) was converted to the standard hydrogen potential (EH) and corrected for temperature (T, degrees Celsius) using the equation of Skoog and West (1976); $EH = EM + 223 + 0.76T$. Total organic nitrogen (TON) was calculated as TKN – NH_4-N, total unreactive phosphorus (TUP) as TP – TRP, and TN as TKN + NO_3-N. All analyses include fine particles (<80 µm) and dissolved fractions.

Sediment cores were collected at the end of May in 1987 and 1988 (Fig. 1). A Plexiglas tube (10 cm in diameter) was inserted by hand to approximately 20 cm depth into the sediments and the extracted sediment was sectioned into 5-cm segments. Sediment TP and TKN were determined as outlined in Ontario Ministry of the Environment (1981).

Water and Nutrient Budget

A general water budget equation for the beaver pond is

$$(1)\quad P + U + \sum^{n} S_i - E - S_o \pm \Delta W = \pm e\,.$$

Inputs include stream inflows (S_i), precipitation depth (P), and ungauged runoff (U) comprising both subsurface and diffuse surface flow from ungauged areas adjacent to the pond. Outputs include stream outflow (S_o), evapotranspiration (E), and change in storage (ΔW). The inputs should balance outputs plus or minus measurement errors (e). In determining water storage the flooded area of the pond was assumed to be constant with depth. Potential evapotranspiration (E) was estimated from Thornthwaite's (1948) equation. All runoff from the base of each tributary stream catchment was assumed to be surface stream flow. Deep groundwater inputs and outputs were assumed to be negligible, due to the impermeable nature of the bedrock.

For this study, waterborne nutrient retention (RT) was calculated from inputs which include bulk atmospheric deposition (P), stream inflow (S_i), unchannelized or ungauged inflows (U), and outputs as stream outflow (S_o):

$$(2)\quad \pm RT = P_i + \sum^{n} S_i + U_i - S_o\,.$$

Both wet precipitation and dry deposition are incorporated into P. Gas fluxes (e.g., dentrification) and weathering inputs are not included; thus, the waterborne nutrient budget is emphasized.

Atmospheric deposition was determined as described in Dillon et al. (1988). Reactive P measurements in bulk deposition were previously determined to be 34 ± 50% of the TP deposition (Dillon and Reid 1981).

Stream load was determined by integrating the estimated daily average discharge over each sampling period and multiplying the total volume of water by the nutrient concentration at the midpoint of each time interval (Scheider et al. 1979). Nutrient loads from adjacent ungauged areas (U) were determined from the mean monthly, volume-weighted concentration of three nearby upland streams multiplied by prorated monthly runoff volume. Annual budgets were determined by addition of the monthly budgets for the hydrologic year June 1 to May 31. Seasonal budgets were determined by addition of the months June to August (summer), September to November (fall), December to February (winter), and March to May (spring). TN and TP storage in the sediments was estimated from the average

TABLE 1. Seasonal and annual water (mm) and Cl (mg/m^2) balance ±1 SD for Harp 4 beaver pond for the 1987–88 hydrologic year. A negative balance represents inputs < outputs, and a positive value represents inputs > outputs.

	Input			Output		Evapotranspiration	
	Stream flow	Ungauged flow	Precipitation	Stream flow	Change in storage	Balance (in–out)	Calculated (Thornthwaite)
Water							
Summer	66 ± 7	1 ± 0	182 ± 22	109 ± 3	−90 ± 24	230 ± 33	275
Fall	449 ± 46	25 ± 8	235 ± 30	645 ± 18	+96 ± 25	−32 ± 63	100
Winter	1547 ± 124	212 ± 64	301 ± 37	2032 ± 48	−96 ± 25	124 ± 154	0
Spring	3890 ± 379	825 ± 245	205 ± 25	4894 ± 142	+124 ± 33	−98 ± 475	86
Annual	5952 ± 401	1063 ± 253	923 ± 58	7680 ± 151	+34 ± 12	224 ± 504	461
Cl							
Summer	0.04 ± 0.01	<0.01 ± <0.01	0.03 ± <0.01	0.04 ± <0.01	—	0.02 ± 0.01	
Fall	0.31 ± 0.04	0.01 ± 0.01	0.05 ± 0.01	0.19 ± 0.01	—	0.18 ± 0.04	
Winter	1.08 ± 0.10	0.12 ± 0.05	0.08 ± 0.01	1.24 ± 0.07	—	0.04 ± 0.13	
Spring	1.63 ± 0.18	0.33 ± 0.12	0.04 ± 0.01	2.24 ± 0.14	—	−0.24 ± 0.26	
Annual	3.06 ± 0.20	0.46 ± 0.13	0.2 ± 0.02	3.71 ± 0.16	—	<0.01 ± 0.29	

chemical content multiplied by the estimated bulk density for each sediment subsample.

Absolute retention (RT) of the beaver pond was calculated as RT = (total inputs – total outputs)/(pond area) and percent retention as %RT = ((total inputs – total outputs)/total inputs × 100.

Error Estimates

The variance of water budget calculations was calculated to obtain the standard deviation (Winter 1981):

$$(3)\quad S_p^2 + S_U^2 + \sum^{n} S_{S_i}^2 + S_E^2 + S_{S_o}^2 + S_{\Delta W}^2 = S_T^2$$

where n is the number of inflow streams (S_i) and (S_T) is the standard deviation of the total monthly water budget. To obtain S_T^2, total monthly water volumes were multiplied by their associated fractional error (CV) and then squared and summed. The variances of all products in this study were approximated as (Mood et al. 1974)

$$(4)\quad \mathrm{VAR}(X,Y) \simeq u_x^2\mathrm{VAR}(Y) + u_y^2\mathrm{VAR}(X) + \mathrm{VAR}(Y)\mathrm{VAR}(X)\,.$$

VAR(X) and VAR(Y) are the product of the water volume or concentration multiplied by the fraction error (CV) and then squared. S_T^2 for each of the nutrient retention estimates was calculated using Eq. 3. To obtain S_T^2 for TN, TON, total inorganic nitrogen (TIN), and TUP retention, variance estimates associated with the parameters used to calculate each mass were summed. The variance associated with nutrient mass was determined for each sampling time interval and summed to produce either seasonal or annual values.

Errors associated with daily and monthly stream discharge measurements range from 18 to 73% for mean daily stream discharge (Devito 1989). Based on comparisons of several tributary stream catchments in the study area, the errors in estimating monthly discharge volume by linear integration and by prorating unit runoff are estimated at ±27 and ±30%, respectively (Devito 1989). Errors associated with measuring precipitation were not determined directly. Errors based on equipment used and the rain fall patterns in this area are assumed to be ±21%/mo (Winter 1981). The range of uncertainty for determining stage for the pond was ±2 mm. The CV associated with estimating the area of the pond from airphotos was assumed to be ±10%.

Analytical and sampling errors associated with determining stream water and bulk deposition chemistry of discrete samples ranged from 2 to 18% (Devito 1989). Errors associated with volume-weighted concentrations are assumed equivalent to analytical and sampling errors.

The errors associated with each component of the budgets were assumed to be random and normally distributed. Unmeasured and systematic errors have not been included in the error analyses. The following variance estimates, therefore, must be considered as only the precision of water and nutrient budget estimates, and actual errors are probably greater than indicated (Devito 1989).

Results

Water and Waterborne Nutrient Budgets

Annual inputs and outputs of water and Cl for 1987–88 in the beaver pond roughly balanced using only runoff and precipitation (Table 1). This fact and the results of a study by McDonnell and Taylor (1987) suggest that deep groundwater fluxes are limited in the upper reaches of Harp 4 subcatchment. On an annual basis the major input for water was via runoff, with precipitation contributing <15%. Potential rates of evapotranspiration and change in storage were minor outputs representing <10 and <1%, respectively. The relative contribution of each component varied seasonally. Positive retention of water and Cl occurred during the summer and winter with negative retention occurring during the spring (Table 1). Precipitation, potential evapotranspiration, and change in storage were dominant components of the summer budget. Runoff increased in

TABLE 2. Waterborne P input, output, and retention estimates (mg P/m^2 ±1 SD) for the 1987–88 hydrologic year for Harp 4 beaver pond.

	Input					Retention	
P form	Stream flow	Ungauged flow	Precipitation	Total	Output: Stream flow	Absolute (in–out)	Relative %
TRP							
Summer	1.8 ± 0.2	0.0 ± 0.0	1.8 ± 0.7	3.6 ± 0.7	0.5 ± <0.1	3.1 ± 0.7	86
Fall	1.2 ± 0.2	<0.1 ± <0.1	1.2 ± 0.4	2.5 ± 0.5	0.6 ± 0.1	1.8 ± 0.5	72
Winter	5.5 ± 1.0	0.2 ± 0.1	0.9 ± 0.3	6.6 ± 1.0	2.0 ± 0.3	4.6 ± 1.1	70
Spring	9.7 ± 1.7	0.7 ± 0.3	1.5 ± 0.5	11.8 ± 1.8	4.0 ± 0.7	7.9 ± 2.0	67
Annual	18.2 ± 2.0	0.9 ± 0.3	5.4 ± 1.0	24.5 ± 2.2	7.1 ± 0.7	17.4 ± 2.3	71
TUP							
Summer	4.8 ± 1.0	<0.1 ± <0.1	3.9 ± 1.0	8.7 ± 1.4	6.3 ± 0.5	2.4 ± 1.5	27
Fall	8.8 ± 1.5	0.2 ± 0.2	2.6 ± 0.7	11.6 ± 1.6	14.1 ± 0.9	−2.5 ± 3.4	−22
Winter	12.6 ± 2.6	1.6 ± 1.6	1.9 ± 0.5	16.1 ± 3.1	28.4 ± 1.7	−12.3 ± 3.5	−76
Spring	42.4 ± 6.2	6.3 ± 6.3	3.1 ± 0.8	51.8 ± 8.9	68.7 ± 4.5	−16.9 ± 9.9	−33
Annual	68.6 ± 6.9	8.1 ± 6.5	11.5 ± 1.6	88.2 ± 9.6	117.5 ± 4.9	−29.3 ± 10.8	−33
TP							
Summer	6.6 ± 1.0	<0.1 ± <0.1	5.7 ± 0.8	12.3 ± 1.3	6.8 ± 0.5	5.5 ± 1.3	45
Fall	10.1 ± 1.5	0.2 ± 0.2	3.8 ± 0.5	14.1 ± 1.6	14.7 ± 0.9	−0.6 ± 1.8	−4
Winter	18.0 ± 2.4	1.8 ± 1.6	2.7 ± 0.4	22.5 ± 2.9	30.3 ± 1.7	−7.8 ± 3.4	−34
Spring	52.1 ± 5.9	6.9 ± 6.3	4.5 ± 0.6	63.5 ± 8.7	72.7 ± 4.5	−9.2 ± 9.7	−14
Annual	86.8 ± 6.6	8.9 ± 6.5	16.7 ± 1.2	112.4 ± 9.3	124.5 ± 4.9	−12.1 ± 10.5	−11

TABLE 3. Waterborne N input, output, and retention estimates (g N/m^2 ± 1 SD) for the 1987–88 hydrologic year for Harp 4 beaver pond.

	Input					Retention	
N form	Stream flow	Ungauged flow	Precipitation	Total	Output: Stream flow	Absolute (in–out)	Relative %
NH_4-N							
Summer	<0.01 ± <0.01	0.01 ± <0.01	0.07 ± 0.01	0.08 ± 0.01	0.01 ± <0.01	0.07 ± 0.01	87
Fall	0.03 ± 0.01	0.01 ± <0.01	0.10 ± 0.01	0.13 ± 0.01	0.05 ± 0.01	0.08 ± 0.02	62
Winter	0.13 ± 0.02	0.01 ± 0.01	0.09 ± 0.01	0.22 ± 0.02	0.24 ± 0.03	−0.02 ± 0.04	−9
Spring	0.18 ± 0.03	0.01 ± 0.03	0.10 ± 0.01	0.29 ± 0.04	0.48 ± 0.06	−0.19 ± 0.07	−66
Annual	0.34 ± 0.03	0.02 ± 0.03	0.36 ± 0.02	0.72 ± 0.05	0.78 ± 0.06	−0.06 ± 0.08	−8
NO_3-N							
Summer	<0.01 ± <0.01	0.01 ± <0.01	0.09 ± 0.01	0.10 ± 0.01	<0.01 ± <0.01	0.10 ± 0.01	100
Fall	0.04 ± <0.01	<0.01 ± <0.01	0.15 ± 0.02	0.18 ± 0.02	0.07 ± 0.01	0.11 ± 0.02	61
Winter	0.13 ± 0.01	0.02 ± 0.01	0.20 ± 0.02	0.35 ± 0.03	0.18 ± 0.01	0.17 ± 0.03	49
Spring	0.33 ± 0.03	0.06 ± 0.07	0.14 ± 0.02	0.53 ± 0.07	0.50 ± 0.04	0.03 ± 0.08	6
Annual	0.50 ± 0.03	0.08 ± 0.07	0.58 ± 0.04	1.16 ± 0.08	0.75 ± 0.04	0.41 ± 0.09	35
TON							
Summer	0.06 ± 0.01	0.01 ± <0.01	0.06 ± 0.02	0.12 ± 0.02	0.06 ± <0.01	0.05 ± 0.022	46
Fall	0.23 ± 0.03	0.01 ± <0.01	0.02 ± 0.02	0.26 ± 0.03	0.31 ± 0.01	−0.06 ± 0.04	−26
Winter	0.41 ± 0.05	0.05 ± 0.05	0.03 ± 0.02	0.49 ± 0.07	0.73 ± 0.04	−0.24 ± 0.08	−50
Spring	0.90 ± 0.11	0.12 ± 0.06	0.04 ± 0.02	1.06 ± 0.13	1.34 ± 0.08	−0.28 ± 0.15	−27
Annual	1.60 ± 0.13	0.18 ± 0.08	0.15 ± 0.04	1.93 ± 0.15	2.44 ± 0.09	−0.51 ± 0.18	−26
TN							
Summer	0.06 ± 0.01	0.01 ± <0.01	0.22 ± 0.02	0.28 ± 0.02	0.08 ± <0.01	0.20 ± 0.02	71
Fall	0.29 ± 0.03	0.01 ± <0.01	0.26 ± 0.02	0.56 ± 0.04	0.43 ± 0.01	0.13 ± 0.04	23
Winter	0.66 ± 0.05	0.07 ± 0.05	0.33 ± 0.03	1.06 ± 0.08	1.15 ± 0.03	−0.09 ± 0.08	−8
Spring	1.40 ± 0.11	0.20 ± 0.09	0.28 ± 0.03	1.88 ± 0.14	2.32 ± 0.07	−0.44 ± 0.16	−23
Annual	2.41 ± 0.13	0.28 ± 0.10	1.09 ± 0.05	3.78 ± 0.17	3.98 ± 0.08	−0.20 ± 0.19	−5

TABLE 4. Annual waterborne nutrient retention ($g \cdot m^{-2} \cdot yr^{-1}$ ±1 SD of budget error) for Harp 4 beaver pond. For the 5-yr average, ±1 SD about the mean ($\bar{x}$).

Year	TP	NH_4-N	NO_3-N	TON	TN
1983–84[a]	0.010 ± 0.023	0.007 ± 0.070	0.461 ± 0.099	−0.320 ± 0.430	0.140 ± 0.440
1984–85	0.014 ± 0.028	−0.258 ± 0.147	0.104 ± 0.147	−0.539 ± 0.549	−0.703 ± 0.549
1985–86	−0.080 ± 0.036	−0.193 ± 0.147	0.381 ± 0.119	−1.457 ± 0.593	−1.269 ± 0.587
1986–87	0.009 ± 0.025	0.038 ± 0.117	0.322 ± 0.090	−0.239 ± 0.405	0.122 ± 0.403
1987–88	−0.012 ± 0.010	−0.062 ± 0.078	0.399 ± 0.091	−0.535 ± 0.179	−0.196 ± 0.186
$\bar{x}$ ± 1 SD	−0.012 ± 0.039	−0.094 ± 0.128	0.333 ± 0.137	−0.618 ± 0.487	−0.381 ± 0.602
Range % RT	−34 to 7	−37 to 6	12 to 54	−52 to −11	−27 to 4

[a]From Devito et al. (1989).

TABLE 5. Runoff, water residence time, and P and N export and import for the 1987–88 hydrologic year for Harp 4 beaver pond.

	Period						
	Annual 1987–88	Summer and fall	Winter and spring	Apr. 1988	Apr. 1–10, 1988	Dec. 10–16, 1987	Jan. 31 to Feb. 6, 1988
No. of days	365	182	183	30	10	7	7
Runoff (1×10^6 L)	293	29	264	132	80	17	13
(% annual)		(10%)	(90%)	(45%)	(27%)	(6%)	(4%)
Water residence time (d)	47	242	26	9	5	16	21
TP (g)							
Export	4744	820	3294	1503	846	248	220
		(17%)	(83%)	(32%)	(18%)	(5%)	(5%)
Import	4284	1005	3280	1339	—	—	—
		(23%)	(77%)	(33%)			
TN (kg)							
Export	151	19	132	53	33	9	9
		(13%)	(87%)	(35%)	(22%)	(6%)	(6%)
Import	144	32	112	41	—	—	—
		(22%)	(78%)	(29%)			

importance and represented the major input and output during the winter and spring months.

Annual retentions of dissolved plus fine particulate TP and TN were low with absolute retention less than the budget uncertainties in 1987–88 (Tables 2 and 3). The beaver pond transformed P and N by retaining TRP (71%) and NO_3-N (35%) with a concomitant release of unreactive P (−33%) and organic N (−26%). There was no significant retention of NH_4-N (−8%). Although there is some variation between years, TP and TN retention was generally less than budget uncertainties over the 5-yr period 1983–84 to 1987–88 (Table 4). All budgets prior to 1987–88 were calculated by prorating unit runoff for stream (S_i and S_o) discharge; as a result, they have a greater error associated with them.

Consistent seasonal trends in P and N retention were observed in the beaver pond during 1987–88 (Tables 2 and 3) related to the 4 yr previous to 1987–88 (data not shown). Generally, P and N were retained during the summer months and released during the winter and spring. Relative retention of TUP and TON was variable with the greatest negative retention occurring during the winter. TRP and NO_3-N were exceptions. NO_3-N was retained during all seasons except during spring where inputs approximated outputs. Relative retention of TRP was high in all seasons with an increase in absolute retention with an increase in inputs.

The absolute input and output of nutrients varied seasonally (Table 5). The majority of the runoff and flux of TP and TN occurred during the winter and early spring and was confined to only a few rain and snowmelt events. During April 1988, 29–35% of the annual input and output of TP and TN of the pond occurred.

Sediment P and N Content and Burial

Typically, 7–12 cm of sediment had been laid down at the coring sites since the pond was established (Table 6). Based on an accumulation period of 20–27 yr, a net annual burial rate of 0.15–0.55 g $P \cdot m^{-2} \cdot yr^{-1}$ and 2.5–6.6 g $N \cdot m^{-2} \cdot yr^{-1}$ was estimated from P and N content of sediment (including forest litter) above the forest floor.

Pond Hydrology and Chemistry

Discharge varied over the year, with low base flow through the summer and peak discharge during snowmelt in March and April (Fig. 2a). Discharge peaks occurring through late fall and winter were a result of snowmelt, associated with rain, where much of the accumulated snowpack was lost.

Water levels in the pond varied seasonally in response to rainfall, evapotranspiration, and upland runoff (Fig. 2a). The lowest water levels were observed during late summer. The actual water storage in the pond was small. A 20-cm rise during the fall represented only about 2 cm of runoff from the sur-

TABLE 6. P and N sediment content and estimated accumulation rate ±1 SD of the mean core samples, calculated as total mass above a given depth divided by the age at that depth.

		TP (g/m^2)			TN (g/m^2)		
Year	No of cores	Top 15 cm	Above old forest floor	Estimated burial rate ($g \cdot m^{-2} \cdot yr^{-1}$)	Top 15 cm	Above old forest floor	Estimated burial rate ($g \cdot m^{-2} \cdot yr^{-1}$)
1987	5	17.1 ± 11.4	7.2 ± 4.5	—	168 ± 63	93 ± 36	—
1988	8	16.7 ± 8.5	7.7 ± 2.8	—	150 ± 44	103 ± 30	—
Pooled	13	16.8 ± 9.3	7.5 ± 3.4	0.15–0.55[a]	157 ± 50	99 ± 32	2.5–6.6[a]

[a]Based on a sediment age of 20–27 yr and upper and lower confidence interval.

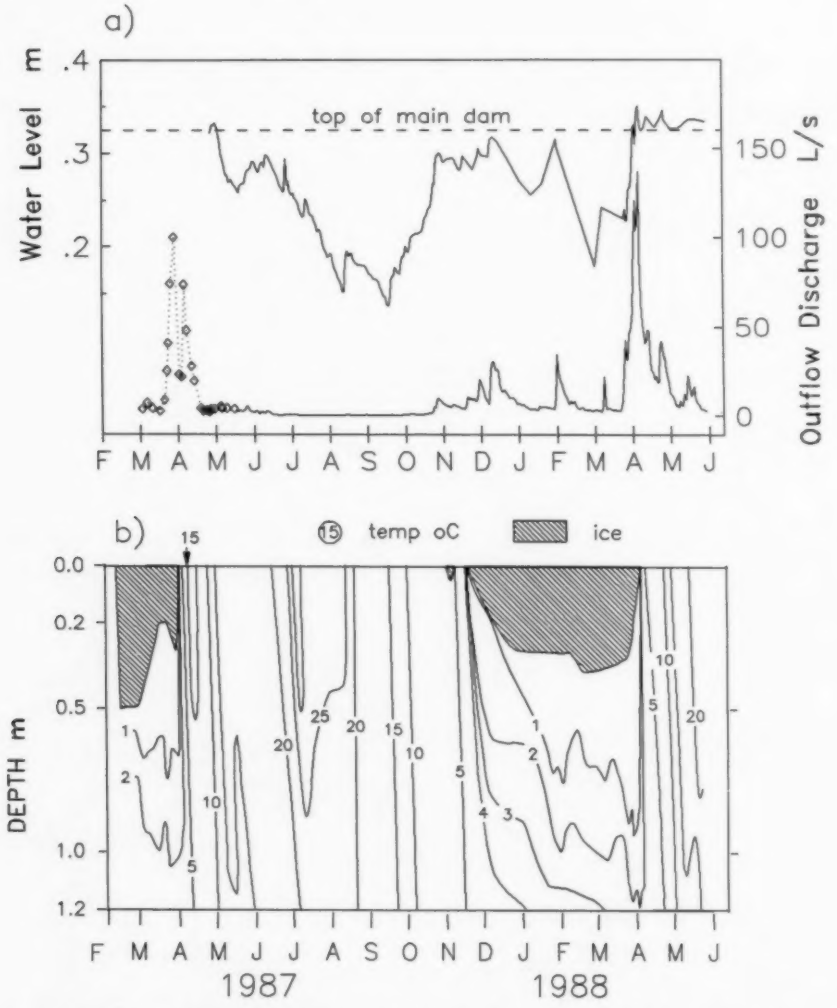

FIG. 2. (a) Pond water level and outflow discharge and (b) temperature profile at site 1 of the beaver pond from March 1, 1987, to May 31, 1988. From March to May 1987, the outflow discharge was measured at discrete periods (broken line).

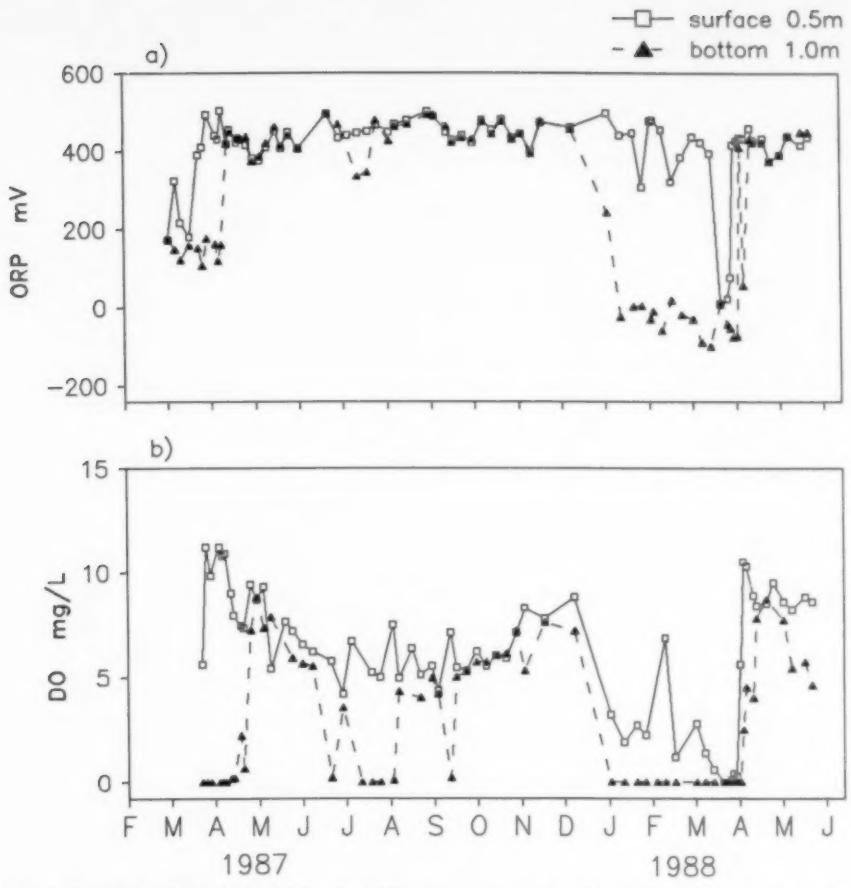

FIG. 3. Seasonal variation in (a) ORP and (b) DO concentrations of surface and bottom water at site 1 of the beaver pond from March 1, 1987, to May 31, 1988.

rounding catchment. Water levels in the pond responded rapidly to increases in runoff with peaks in water level coinciding with outflow hydrographs. Water levels exceeded the main dam height during both the 1987 and 1988 spring snowmelt. The annual residence time of water for 1987–88 in the existing pond was 47 d (Table 5). This compares with 6 h for the primary stream if it had the same channel structure as the outflow. The residence time of water varied seasonally, being 242 d for summer–fall and 26 d for winter–spring. During peak spring melt, April 7, 1988, the residence time of water in the pond was less than 1 d.

Water temperatures of the pond varied seasonally from near 0°C in late winter to 30°C in midsummer (Fig. 2b). There was little or no thermal stratification of the pond water through the ice-free season. Thermal stratification began with ice formation and was maintained through the winter until peak spring discharge and breakup of the ice. Dissolved oxygen (DO) concentrations sporadically dropped below detection in the bottom water during the ice-free season, but generally the ORP and DO concentrations of both the surface and bottom water were high (Fig. 3). The bottom water became anoxic following winter ice cover. The DO concentrations in the surface water (just below the ice) declined during the ice-cover period. The entire water column became anoxic by late March, just prior to the spring melt.

Temporal and depth variations in P and N concentrations were influenced by periods of ice cover and thermal stratification (Fig. 4 and 5). TP and TN concentrations were slightly higher during the summer, while minimum TN and TP concentrations occurred after increased runoff during the fall and spring. Concentrations near the surface generally remained low through the winter. The concentrations increased in the bottom water to near maximum annual values during early spring when anoxic conditions existed for an extended period of time. TON and TUP were the predominant forms of N and P. Inorganic N and TRP remained near detection limits throughout the ice-free season. High NO_3-N concentrations occurred in the surface water during periods of snowmelt and increased discharge. TRP concentrations increased in the bottom during winter anoxic conditions. High levels of NH_4-N (>500 µg/L) were observed in the bottom and eventually the surface waters in the beaver pond during the winter and early spring. Following spring snowmelt, concentrations in the water column were at or near minimum annual values.

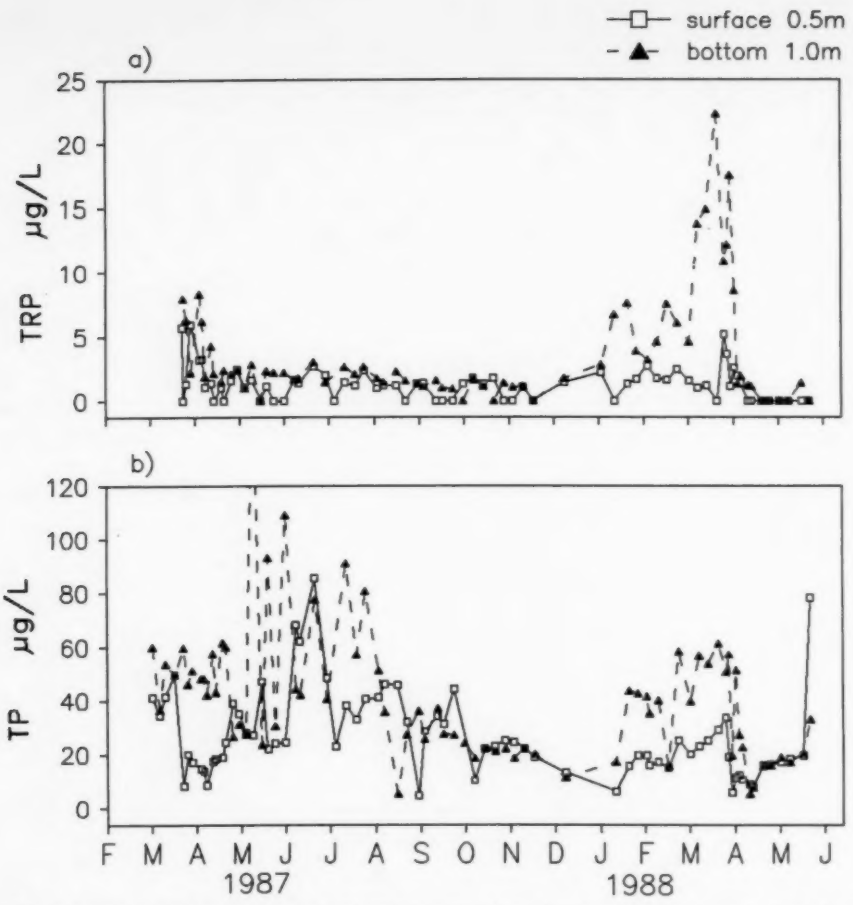

FIG. 4. Seasonal variation in (a) TP and (b) TRP concentrations of surface and bottom water at site 1 of the beaver pond from March 1, 1987, to May 31, 1988.

Monthly Retention in Relation to ORP and Discharge

There was an inverse relationship between monthly retention of TP and TN and runoff ($r = -0.70$ and -0.82) for March 1987 to May 1988 (Fig. 6). There was considerable scatter in the runoff versus RT relationships, which appears to be due to interaction with temperature and redox condition of the pond water. There was a weak linear relationship between monthly retention and average water temperature ($r = 0.53$ and 0.55). Low average monthly ORP was associated with negative retention of TP and TN ($r = 0.48$ and 0.43) in the pond during the winter months. There were 4 mo with average ORP near or below 200 mV in which TP and TN retention was much less than ice-free months with similar or greater discharge. The lowest monthly retention in the beaver pond occurred during December 1987 and March 1987 for TP and TN, respectively. The runoff volume during these months was less than half the maximum observed monthly runoff over the past 4 yr.

Discussion

Annual P and N Retention

Low annual TP and TN retention in the beaver ponds studied is a relatively long-term phenomenon, as no significant retention was observed over 5 yr. It appears that cycling of P and N within the pond has reached steady state, or the retention rate may be too low to be detected when compared with the large influxes and effluxes.

Low annual TP and TN retention may commonly occur in beaver ponds situated throughout the Precambrian shield landscape. Little retention of waterborne N was measured in a second-order beaver pond on the Precambrian Shield in Quebec (Naiman and Melillo 1984). Although Naiman and Melillo (1984) reported a net accumulation, primarily due to N fixation, 99% of the inputs were via stream throughflow and the total mass balance measured only a 5% TN retention. Devito et al. (1989) reported low TP and TN retention in another pond situated in a small forested catchment near this study site. In a study of 32 forested stream catchments in central Ontario, peat deposits or beaver ponds were suggested to be the likely source of high TP and TON export (Dillon et al. 1991).

Maret et al. (1987) reported a positive retention of TP, NO_3 and TKN during the ice-free season in a beaver pond complex in southeastern Wyoming. The retentive function of beaver ponds may be greater in high-gradient systems with large mineral sediment loads where construction of a dam results in deposition

of that stream load. Maret et al. (1987) found that the beaver pond did not reduce nutrient levels during the summer when mineral particulate load and deposition were reduced.

The primary role of the beaver pond we studied is to transform inorganic forms of N and P into organic forms which are subsequently transported downstream. Low annual retention and net transformation of inorganic to organic TP and TN have been reported in other geographically diverse stream and riparian wetland ecosystems (Meyer et al. 1981; Elder 1985).

The need for error estimates is paramount in interpretation of budget residuals and is stressed in this study. Elder (1985) reported errors of annual yield estimates for the Apalachicola River wetland system, calculated from the sums of squared component SD, of 5–6% for N and 8–9% for P. Dodds and Castenholz (1988) reported differences between the minimum or maximum from the mean estimates ranging from 67 to 170% of the total N inputs or outputs of a pond. Their estimates were conservative, since variance is propagated additively. The errors for annual estimates of stream nutrient flux in the study pond ranged from 2 to 11% with most SD near 10%. Prorating unit runoff introduces error and this is reflected in the large budget uncertainties for the annual budgets calculated prior to 1987–88.

Error estimates of this study suggest that the difference between inputs and outputs must be about ±20% of the inputs to be considered significant with 95% confidence. Because of the large throughput of water and dissolved material, absolute rates of retention may be difficult to detect due to inherent uncertainties of the budgets.

Sediment Burial

The burial rates for the beaver pond are slightly lower but comparable with burial rates of 26 and 7 g $N \cdot m^{-2} \cdot yr^{-1}$ reported for a beaver-inhabited spring in Oregon (Dodds and Castenholz 1988) and a beaver pond complex in Quebec (Naiman and Melillo 1984). No annual P accumulation rates in beaver ponds have been reported. Although there are large uncertainties associated with the burial estimates, long-term sequestering of P and N is suggested.

The burial rates are an order of magnitude greater than the budget error estimates, suggesting that the large standing stock of P and N in the sediments must be derived from unmeasured inputs. The water volume and chemistry of unchannelized runoff from hill slopes adjacent to the swamp were not measured directly. Ungauged inputs to the pond represented <10% of the total inputs, and a doubling or halving of the inputs results in annual retention that is within 1 SD of the calculated retention. Devito et al. (1989) used empirical models to estimate the litterfall inputs to a number of wetlands in the study area and found these fluxes to be <5% of the total inputs.

Rates of N fixation in similar sediments, based primarily on the ice-free season, range from 0.4 to 6.0 g $N \cdot m^{-2} \cdot yr^{-1}$ and approximate the net burial rates in the study pond (Naiman and Melillo 1984; Dodds and Castenholz 1988). However, rates of dentrification are probably within the rates of N fixation. Molot and Dillon (1993) measured annual dentrification rates, using the N:P ratio method, ranging from 0.8 to 1.2 g $N \cdot m^{-2} \cdot yr^{-1}$ for a number of lakes in the study area. Although the values probably represent potential rates and thus overestimate annual rates, N fixation and dentrification could represent major fluxes to the pond sediments. There are, however, no analogous microbial activities which could account for the large accumulation of P.

It is important to recognize the dynamic nature of beaver ponds and the influence of beaver on the landscape. Estimates of the amount of vegetation required by an individual beaver for growth and maintenance range from 183 to 913 kg/yr (Novak 1987). The number of beaver in a family unit range from three to eight with 7.5 being the average for central Ontario (Novak 1987). With 70% of the annual requirement as woody vegetation (Novak 1987), and a nutrient content of 0.065% TP and 0.987% TN (Lozano 1987), the amount of P and N introduced into the pond by a family of three to eight beaver could range from 0.25 to 3.32 kg P/yr and from 5.42 to 72.1 kg N/yr. It is difficult to estimate the amount of activity in a particular pond because of periodic removal by trapping and natural predation; however, beaver inputs could be very important to the nutrient budget of the pond, representing 6–77 and 4–50% of the P and N inputs, respectively. Indirect inputs by beaver have also been considered important to the N budgets of other beaver ponds (Naiman and Melillo 1984; Dodds and Castenholz 1988).

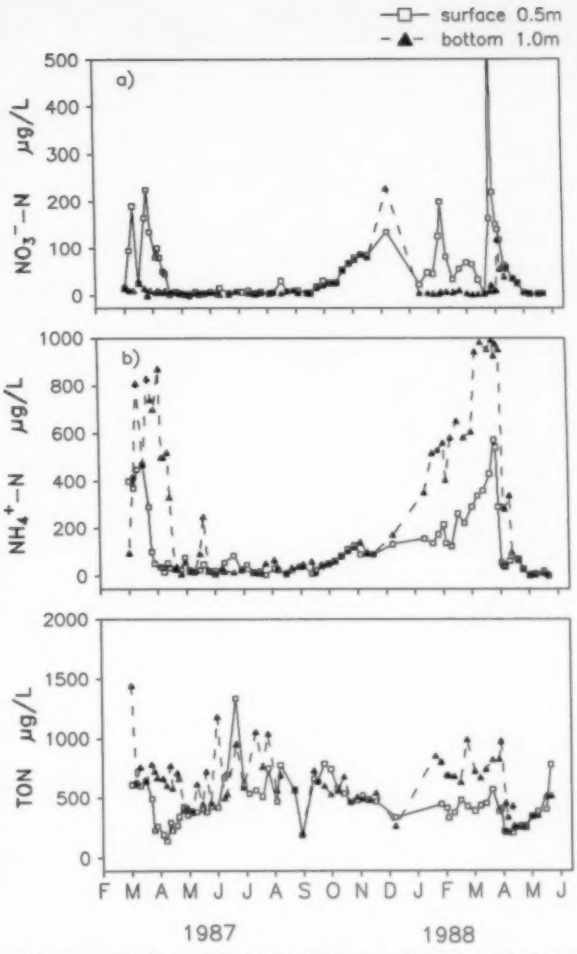

FIG. 5. Seasonal variation in (a) NO_3-N, (b) NH_4-N, and (c) TON concentrations of surface and bottom water at site 1 of the beaver pond from March 1, 1987, to May 31, 1988.

Beaver inputs cannot account for all the sediment pool in the study beaver pond. The beaver flooded 3.8 ha of a forested valley. The P and N contents of aboveground vegetation and soil litter

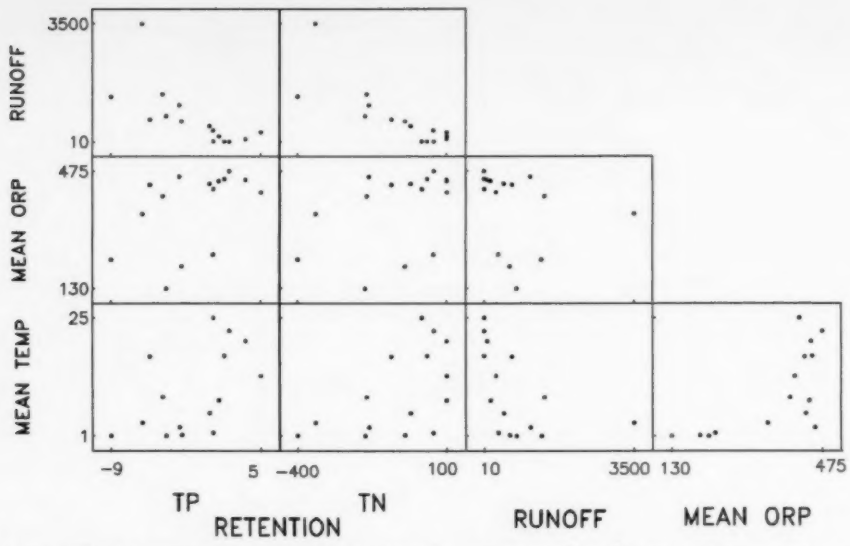

FIG. 6. Pairwise comparison of monthly TP and TN retention ($mg \cdot m^{-2} \cdot mo^{-1}$) with monthly runoff (mm), mean redox potential (mV), and temperature (°C) for the beaver pond for March 1987 to May 1988.

layer of the forested uplands adjacent to the pond range from 2.7 to 7.7 g $P \cdot m^{-2}$ and from 30.4 to 106.2 g $N \cdot m^{-2}$ (Lozano and Parton 1987; Lozano et al. 1987). Initial input of forest litter and vegetation upon flooding can account for the buried P and N pool. Anoxic conditions in the sediments may slow decomposition and a considerable amount of the initially large pool of P and N may still remain.

Although there is considerable uncertainty about these estimated inputs, they suggest that construction of a dam and beaver activity can increase the amount of organic material and mineral soils which are hydrologically linked to the outflow stream. Such linkage has been suggested in other studies (e.g., Naiman et al. 1987). This "reservoir" of P and N may be mobilized, representing a low-rate but long-term source of nutrients to the pond water and downstream.

Influence of Hydrology and Winter Anoxia on P and N Retention

The seasonal, and thus annual, retention in the beaver pond is controlled primarily by hydrology, particularly runoff magnitude and the residence time of water in the beaver pond. Retention is further moderated by nutrient assimilation, and regeneration of P and N from organic sediments, primarily during the relatively long period of anoxia during ice cover.

Gross export and absolute retention of P and N within the beaver pond were primarily controlled by seasonal variations in runoff. Outflow concentrations varied by an order of magnitude at low flow but remained relatively constant with increases in outflow discharge >5 L/s (Devito 1989). Discharge varied over four orders of magnitude; thus, P and N export was directly proportional to stream discharge. Increased gross export of elements as discharge increases has been widely reported (Hill 1988; Dillon et al. 1991).

Episodic events are important in the annual retention and transport of P and N in the study beaver pond. Accumulation of snow redistributed the precipitation of several months into three hydrologic events in which >40% of the annual P and N inputs and outputs to the pond occurred. High nutrient flushing rates restrict nutrient removal from the water column to rapid chemical or biological reactions. Greater than 50% of the annual water and nutrient yield from many temperate and boreal catchments has also been reported to occur during episodic storms or snowmelt (Scheider et al. 1979; Meyer et al. 1981; Maret et al. 1987).

Construction of the beaver dam greatly alters the stream hydrology and resulted in two orders of magnitude increase in the annual residence time of water within the stream reach assuming the primary channel had the same structure as the outflow. This may greatly increase autotrophy in low-order streams (Naiman et al. 1987) and greatly increase nutrient retention within the stream reach. One season's growth of *Potamogeton crispus* and periphyton community in a shallow lake (mean depth 2 m) in south central Ontario incorporated on average 0.9 g P/m^2 and 8.7 g N/m^2 (Ontario Ministry of Environment and Energy 1993). With similar growth rates, P and N uptake by the algal and macrophyte community in the pond would exceed the annual TP and TN inputs to the beaver pond. The low surface concentrations and high retention efficiencies suggest that biotic assimilation may control inorganic P and N retention in the beaver pond, at least during ice-free periods.

An important consideration is the timing of nutrient cycling processes relative to variations in runoff and nutrient transport. Periods of high assimilation rates occur when nutrient transport is low and contributes little to the annual nutrient flux of the beaver pond. High nutrient flushing rates coupled with low water temperature during the winter and early spring resulted in lower N retention efficiencies (–66 to 49%) than would be expected using only the growing season estimates (>85%). Inorganic N inputs during April and the winter storm events ranged from 21 to 58% of the estimated potential periphyton–macrophyte uptake (24 mg $N \cdot m^{-2} \cdot d^{-1}$). The integrated annual estimates almost certainly overestimate actual uptake during the winter and early spring. N input rates appear to exceed macrophyte and

periphyton uptake during spring melt and storm events when a large portion of the annual inputs enters the pond. TRP was efficiently retained during the spring. TRP inputs during April and the winter storm events ranged from 4 to 9% of integrated annual estimates of potential periphyton–macrophyte uptake (2.4 mg $P \cdot m^{-2} \cdot d^{-1}$). Biotic demand for inorganic P is probably greater than inputs during winter and spring snowmelt events and potentially controls inorganic P retention in the pond during high runoff.

The annual retention in inorganic P and N was roughly balanced by net export of organic N and P resulting in low annual TN and TP retention. Flushing of dissolved and fine particulate organic N and P from the pond was evident during the fall and spring melt. Algal and other microbe populations comprise a portion of the fine particulate organic P and N and would be lost from the system during periods of high runoff (Atchue et al. 1983). A portion of nutrients assimilated by the macrophytes will be lost to the water column in the fall following senescence (Ontario Ministry of Environment and Energy 1993).

Significant amounts of P and N may be regenerated from the accumulated organic matter in the pond sediments, which was the major pool of P and N in the study pond. Increased concentrations of dissolved organic carbon (DOC) are associated with organic decomposition (Naiman et al. 1986; Dahm et al. 1987) and were observed during the summer and winter in the study pond (Devito 1989). The source of organics to the sediments in this pond is probably beaver inputs and the forest vegetation and soil that were flooded initially. This would imply that the available pool of organic material could vary between ponds depending on (1) the age of the pond and the amount of organic material accumulated, (2) the activity of the beaver populations, and (3) the catchment morphometry and dam height which determine the amount of land and forest material flooded.

Anaerobic conditions in the study pond during winter ice cover had a strong influence on the concentration of P and N in the beaver pond. This has been shown for many water bodies in northern temperate regions (e.g., Knowles and Lean 1987; Carignan and Lean 1991). The greatest net export of TP and TN occurred during ice cover and anoxic conditions. Anoxia during ice cover resulted in disproportionately greater export relative to runoff and a negative storage of NH_4-N during the winter and early spring. The winter period of high respiration and organic matter oxidation relative to primary production is always followed by extreme runoff conditions during snowmelt. The hydraulic characteristics of the beaver pond are such that most of the incoming P and N and that accumulated in the surface water is flushed from the system, resulting in a net efflux during the spring and low annual retention of P and N.

Alteration of stream hydrology by the beaver dam may facilitate anaerobic conditions of the stream reach. Neither the inflow nor the outflow stream to the study pond became anoxic (Devito 1989). The increased water depth and reduction in water velocity as a result of damming promote ice formation. Solid ice covers forms an efficient barrier to atmospheric oxygen and eliminates wind-induced mixing which occurs during the ice-free period. This barrier, combined with increased water residence time, results in a greater potential for oxygen depletion and the buildup of reduced forms of P and N. However, the occurrence of anoxic conditions under the ice will also depend on the catchment area to pond area ratio and the catchment morphometry which determined the water residence time and the amount of organic matter available for microbial respiration. These factors are likely to vary from pond to pond.

Exports of NH_4-N from the study pond occurred every winter over the past 5 yr, and has been observed in other ponds in the study area (Devito et al. 1989). High concentrations of NH_4-N were observed in fall and winter below a beaver pond in the Adirondack region of New York (Driscoll et al. 1987). The occurrence of highly reduced conditions in beaver ponds may be common, and this may have significant ramifications for the export of nutrients to downstream ecosystems in landscapes with large beaver populations, such as this area of central Ontario.

Differences in nutrient retention efficiencies between beaver ponds and other wetlands reflect differences in the hydrology, which determines the magnitude and rate of nutrient transport as well as influences the physical environment within the pond. This work shows that characterizing how biotic and geochemical cycling and water anoxia vary temporally with the magnitude of runoff is needed in conjunction with a budget approach to develop reliable generalizations of the influence of beaver ponds on the Precambrian Shield landscape.

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Photosynthetic Carbon Assimilation and Allocation by Surface and Deep Phytoplankton in Colpoys Bay (Western Georgian Bay)

Ralph E.H. Smith and Julie A. Maly

Biology Department, University of Waterloo, Waterloo, ON N2L 3G1, Canada

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We used reciprocal shift experiments to test the hypothesis that surface (i.e., epilimnetic) and deep (i.e., metalimnetic) phytoplankton from thermally stratified Colpoys Bay (Georgian Bay) would differ in their photosynthetic and biosynthetic patterns. A deep chlorophyll maximum existed in the metalimnion during the first half of the stratified season. Surface phytoplankton always had higher light-saturated rates of photosynthesis (P_m^B) and photoadaptation parameters (I_k) than did deep phytoplankton, but most of the difference was due to the proximate effect of temperature on P_m^B, and not to true photoadaptation. Neither sensitivity to photoinhibition (limited) nor light-utilization efficiency (α) varied between surface and deep populations. Depth-related differences in photosynthate allocation to protein, total lipid, neutral lipid, and phospholipid were significant according to ANOVA but were nonetheless small compared with other sources of variation. Photosynthetic rates were generally low, while allocation to polysaccharide and neutral lipid was large, suggesting a relatively poor physiological condition of the phytoplankton. Short-term dynamics (days to weeks) of water masses in Colpoys Bay, and probably many other nearshore Great Lakes locations, appear sufficient to preclude strong vertical differentiation of phytoplankton physiology.

Nous nous sommes servis d'expériences de remplacement réciproques pour tester l'hypothèse selon laquelle le phytoplancton de surface (épilimnique) et le phytoplancton profond (métalimnique) dans les eaux à stratification thermique de la baie Colpoys (baie Georgienne) présenteraient des différences dans le régime de la photosynthèse et de la biosynthèse. Un maximum profond de chlorophylle existait dans le métalimnion pendant la première moitié de la saison de stratification. Le phytoplancton de surface était toujours supérieur au phytoplancton profond en ce qui concerne les taux de saturation lumineuse dans la photosynthèse (P_m^B) et les paramètres de photoadaptation (I_k), mais la plus grande partie de la différence était due à l'effect immédiat de la température sur P_m^B et non à une véritable photoadaptation. On n'a pas observé de variation entre les populations superficielles et profondes en ce qui concerne la sensibilité à la photoinhibition (limitée) ni dans l'efficience de l'utilisation de la lumière (α). Les différences liées à la profondeur dans l'allocation du photosynthétat aux protéines, aux lipides totaux, aux lipides neutres et aux phospholipides étaient significatives selon l'ANOVA, mais restaient faibles comparées aux autres sources de variation. Les taux de photosynthèse étaient généralement faibles, tandis que l'allocation aux polysaccharides et aux lipides neutres était forte, ce qui semble indiquer une condition physiologique relativement mauvaise du phytoplancton. La dynamique à court terme (jours à semaines) des masses d'eau dans la baie Colpoys, et probablement de nombreuses autres zones littorales des Grands Lacs, semble suffisante pour empêcher une forte différenciation verticale dans la physiologie du phytoplancton.

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Natural bodies of water with high clarity and persistent vertical density stratification commonly display a non-uniform vertical profile of chlorophyll *a* concentration, with a maximum below the surface mixed layer. Deep chlorophyll maxima (DCM), as they are often termed, are known from both fresh and salt water (e.g., Fee 1976; Cullen 1982; Fahnenstiel and Glime 1983; Moll et al. 1984; Pick et al. 1984; Fahnenstiel and Scavia 1987a; Platt et al. 1991). The DCM complicates the modelling of primary production, particularly at the synoptic scale required to characterize large water bodies (Fee 1976, 1980; Platt et al. 1991). It also frequently represents a unique assemblage and concentration of algal biomass for consumers, distinct from the surface community (Fahnenstiel and Scavia 1987b).

The photosynthetic characteristics of the phytoplankton may also vary between the surface mixed layer and deeper strata, due partly to photoadaptive changes in pigment contents and photosynthetic apparatus (e.g., Platt et al. 1982; Gallegos et al. 1983; Harding et al. 1985; Fahnenstiel et al. 1989). This poses an additional obstacle to synoptic estimates of primary production, which cannot in general depend on in situ methods but must instead use models of photosynthesis as a function of irradiance (e.g., Fee 1980; Platt et al. 1982). The effort required to routinely determine the depth profile of photosynthesis–irradiance parameters is prohibitive, so it is highly desirable to determine whether depth-related differences can reasonably be ignored, or adequately predicted on the basis of simpler variables (see Fee 1980; Platt et al. 1991). Some "photoadaptive" differences, such as a higher light-saturated rate of photosynthesis for surface than for deep populations, can potentially be explained and predicted simply from temperature. Other changes, such as altered values for the efficiency of light utilization at limiting irradiance (e.g., Fahnenstiel et al. 1989), are not always so easily predicted a priori. There are still few relevant measurements of photo-

synthetic characteristics and their depth dependency for the phytoplankton of the Laurentian Great Lakes, and other large oligotrophic lakes.

The different conditions of life for deep versus surface phytoplankton (Cullen 1982; Moll and Stoermer 1982; Fahnenstiel et al. 1984) may also be expected to cause differences in the biosynthetic patterns of the algae, with consequences for food quality for consumers and transport of contaminants. For example, the lower irradiance and possibly greater nutrient availability in some deep communities (Moll and Stoermer 1982; Fahnenstiel et al. 1984) may favour relatively more production of protein, and less of polysaccharide and lipid, than in the surface communities (Healey and Hendzel 1980; Morris 1981; Smith and Geider 1985). The altered spectral quality of light may also enhance protein synthesis in deep populations (Pick and Cuhel 1986). Differences in species composition between surface and deep communities, which are quite variable among different aquatic systems (e.g., Fahnenstiel and Glime 1983; Pick et al. 1984; Fahnenstiel and Scavia 1987b), may be an additional cause of vertical differentiation of biosynthetic patterns.

Relatively simple predictions of protein or polysaccharide synthesis may be possible from abiotic variables, such as photoperiod or temperature (e.g. Lean et al. 1989), but there are too few data for deep populations to know whether they will generally conform to the behaviour of surface populations. Lipid synthesis may be somewhat less variable than protein or polysaccharide synthesis in natural phytoplankton communities, and is also predictable to some degree from photoperiod and temperature in freshwater phytoplankton (Wainman and Lean 1992). Total lipid, as usually measured, comprises functionally diverse classes of molecules that are expected to respond differently to major environmental variables (Parrish 1987; Lombardi and Wangersky 1991; Smith and D'Souza 1993). For example, neutral lipids classically accumulate in response to nutrient limitation in algal cultures, while the relative contribution of polar lipids decreases (e.g., Lombardi and Wangersky 1991). Relevant data for natural communities of microalgae are still few (Smith and D'Souza 1993), yet the patterns of lipid class synthesis may constitute a useful addition to our stock of methods for assessing the physiological condition of algae in nature. They may also lead to better models for predicting lipid synthesis by natural phytoplankton communities.

Most study of deep plankton communities, as compared with epilimnetic communities, in freshwater has been done either in small lakes or well offshore in the Great Lakes. The large nearshore zone of the Great Lakes is comparatively neglected. The goal of the present work was to determine whether surface and deep phytoplankton at a strongly stratified nearshore site in oligotrophic western Georgian Bay differed significantly in their photosynthetic and biosynthetic patterns, and to what extent the differences could be explained by proximate effects of light and temperature, rather than true physiological differentiation. The site (Colpoys Bay) was close inshore but had many of the characteristics of Georgian Bay proper (Munawar 1988). Water depth and stratification, clarity, phytoplankton biomass and species composition and major nutrient chemistry in Colpoys Bay all resemble those of central Georgian Bay (Maly 1992). Like many other nearshore locations, however, Colpoys Bay experiences significant physical perturbation associated with seiche activity. We hypothesized that a DCM should nonetheless form in the clear waters of Colpoys Bay and that some degree of vertical differentiation in phytoplankton physiology should occur during the stratified season.

Materials and Methods

Study Site and Sampling

The study site (44°50′N, 81°2′W) in Colpoys Bay, southwestern Georgian Bay, Lake Huron, was approximately 300 m offshore in 65 m of water. Colpoys Bay is moderately deep, highly oligotrophic, and receives relatively little surface runoff (Munawar 1988). The seasonal patterns of thermal stratification, water clarity, particulate matter, and phytoplankton concentrations were studied from late May through mid-October 1991 (Maly 1992). Temperature and water clarity were measured using a Richard Brancker Research Ltd. profiling data logger (model TD-400) with sensors for pressure, temperature, and light transmission (SeaTech Inc. model TR2025, 25-cm pathlength). Samples were pumped from depths of up to 43 m for chlorophyll *a* and phytoplankton analysis. Deeper samples and all samples for physiological experiments were taken with a Van Dorn bottle. Two depths were sampled from both the epilimnion and the metalimnion.

Samples for chlorophyll *a* were filtered in the shore laboratory within a few hours of collection on glass fibre filters (Whatman GF/F) and then frozen pending analysis. Phytoplankton samples were preserved with acid Lugol's solution. Samples for physiological experiments were collected in acid-cleaned polycarbonate containers and transported in coolers to the isotope laboratory in Waterloo. Experiments began within 5.5–6.5 h of sampling.

Physiological Experiments

Two types of experiment were performed on each occasion. In the first, a small-volume light gradient incubator (Lewis and Smith 1983; Smith and Herman 1991) was used to measure the photosynthesis–irradiance (*P–I*) response of each population (surface and deep) by ^{14}C assimilation at its own natural temperature and at the temperature of the other population. The light (from a Kodak ELH tungsten-halogen lamp, colour temperature 3350°K) was adjusted with neutral-density filters to provide irradiance below and above the light saturation point expected from previous reports for phytoplankton in the upper Great Lakes (Fahnenstiel et al. 1989). No acclimation period was provided. The intent was to capture the immediate temperature response of the *P–I* function as it would appear in a typical protocol for incubator measurements of primary production.

For *P–I* experiments, 0.25 ml of ^{14}C-labelled bicarbonate solution (ICN Biomedicals; 6.7×10^{11} Bq/mL) was mixed with 25 mL of sample and the labelled suspension then dispensed in 1.0-mL aliquots into 7-mL borosilicate glass vials in the incubator. After 1 h of incubation, the vials were acidified to pH < 2 to drive off unincorporated $[^{14}C]CO_2$. After 24 h, the samples were assayed for total organic ^{14}C activity by liquid scintillation. Total ^{14}C activity (before acidification) was also determined to verify the nominal added isotope concentration. Samples of the unlabelled suspensions were filtered (Whatman GF/F) and frozen pending analysis for chlorophyll *a*. Carbonate alkalinity was determined by acid titration (American Public Health Association 1989) for subsequent calculation of the mass of carbon assimilated.

The second type of experiment tested the effects of reciprocal shifts in temperature and irradiance on photosynthate allocation

by surface and deep populations. Incubations longer than 4 h are desirable in measurements of allocation (Smith and Geider 1985). We used incubations of 5-6 h in 50-mL polystyrene flasks in temperature-controlled incubators with cool-white fluorescent lighting. Based on previous measurements in Georgian Bay (R. Smith, unpublished data), we set the irradiance for surface and deep populations to 180 and 30 μmol photons·m^{-2}·s^{-1}, respectively, and the temperatures to suit the prevailing in situ conditions at the time of the experiment. Duplicate containers were incubated for each treatment (i.e., each combination of population, temperature, and light).

An aliquot of 185 mL from each population was mixed with 0.75 mL of the ^{14}C solution and then dispensed equally to eight flasks for incubation. Samples were taken immediately for initial total and particulate organic ^{14}C activity. Samples for particulate organic activity (5 mL) were collected on GF/F filters, acidified to drive off residual inorganic carbon, and mixed with scintillation cocktail after 24 h. Samples of 15 mL were also filtered and frozen for later determination of initial activity in end-products and lipid classes. After incubation, samples of 5 mL for particulate organic activity and of 15 mL for end-products and lipid classes were filtered and frozen. All activities were subsequently corrected for the initial, or time-zero, values.

Chlorophyll *a* was determined by fluorometry after extraction for 18–24 h in 90% acetone at –20°C (Smith and D'Souza 1993).

End-product and Lipid Analyses

Determination of end-products followed a variant (Smith and Geider 1985; Smith and D'Souza 1993) of the method of Li et al. (1980). In brief, samples were extracted in 2:1 (v/v) chloroform–methanol, and the soluble material was then separated into an aqueous-methanol phase (termed low molecular weight material, or LMW) and a chloroform phase (total lipid). The chloroform phase was evaporated to dryness under a stream of oxygen-free nitrogen gas and frozen pending analysis of lipid classes. The insoluble material was extracted in 5% trichloroacetic acid (TCA) at 80–90°C for 1 h. The TCA-soluble material corresponds mainly to polysaccharide, and the insoluble material to protein.

To determine the distribution of labelled photosynthate among broadly defined lipid classes, the total lipid extract was dissolved in 1.0 mL of HPLC-grade chloroform and chromatographed by a variation of the method of Yongmanitchai (1991). A subsample of 0.1 mL was taken for direct assay of total lipid activity and the remaining volume loaded to a prepacked column of silica gel (Sep-Pak, Waters Assoc. Ltd.). The sample was then eluted by six solvent phases of increasing polarity: HPLC-grade chloroform + 1% ethanol, 1:1 chloroform–acetone, pure acetone, 2:1 chloroform–methanol, 1:1 chloroform–methanol, and pure methanol. Phase 1 elutes neutral lipids (NL); phases 2 and 3 together are denoted the acetone-mobile polar lipids (AMPL), mainly glycolipids and pigments; phases 4 and 5 elute phospholipids (PL), and phase 6 elutes breakdown products (always negligible in the present samples). Smith and D'Souza (1993) and Yongmanitchai (1991) discussed standardization of the method. Collected eluates and the total lipid subsample were evaporated to dryness and then assayed by liquid scintillation.

Statistical Analyses

The relationship between biomass-specific photosynthesis (P_s^B, grams carbon per gram chlorophyll *a* per hour) and irradiance (I, micromoles photons per square metre per second) was fitted to the function (Platt et al. 1982)

$$(1)\quad P^B = P_s^B(1 - e^{-a})e^{-b}$$

where $a = \alpha I/P_s^B$, and $b = \beta I/P_s^B$. P_s^B is the maximum, light-saturated rate of photosynthesis that would be realized in the absence of photoinhibition. The parameter α is an index of light utilization efficiency and is the initial slope of the *P–I* curve at very low (limiting irradiance. The parameter β is an index of photoinhibition at higher irradiance. If photoinhibition is negligible or absent, β will not be different from zero, and

$$(2)\quad P^B = P_m^B(1 - e^{-a})$$

where P_m^B is the realized maximum rate of photosynthesis. The so-called photoadaptation parameter, I_K (micromoles photons per square metre per second), is an index of the onset of light saturation and is calculated as P_m^B/α. Nonlinear least-squares (both derivative and derivative-free algorithms, Wilkinson 1989) was used to estimate the parameters. Inferences concerning statistical significance of differences were made by comparing 95% confidence intervals (CI) for evidence of overlap.

The effects of experimental treatments on end-product and lipid class allocation were analyzed principally by analysis of variance (ANOVA, Wilkinson 1989), using $p < 0.05$ as the significance level. Because the proportions of the classes analyzed lay mainly between 10 and 60% and the variance appeared reasonably stable when residuals were examined, no transformation was applied to the data before analysis.

Residuals and fitted values from both types of analysis (curve fitting and ANOVA) were compared with the original data to verify interpretations.

Results

Thermal Structure and Phytoplankton Populations

Direct thermal stratification began to form in late June and was well established by early July. The epilimnion had warmed to approximately 20°C on 26 July, when the samples for the first photosynthetic experiments were taken (Fig. 1), while the hypolimnion remained near 4°C. The thickness of the epilimnion varied from about 5 to 33 m on scales of days and weeks throughout the stratified season, averaging 16 m for July and August. The lower limit of the metalimnion varied on similar time scales, from 32 to 52 m, averaging 38 m. Despite the large variability in thickness of epilimnion and metalimnion, direct thermal stratification was found on each of the 21 sampling dates from 5 July until mid-October.

Profiles of light transmission throughout July and early August revealed a relatively transparent epilimnion, with substantially reduced transparency in the metalimnion, consistent with a metalimnetic peak of particle concentrations (e.g., Fig. 1). By mid- to late August, the transmission profiles suggested that the metalimnion was no longer the site of a particle concentration maximum, but perhaps a minimum instead (e.g., Fig. 1). This situation persisted to the end of the stratified period.

Chlorophyll *a* concentrations were higher in the metalimnion than the epilimnion for most of July and early August (Fig. 2). By late August, there was little difference between the layers, and the metalimnetic chlorophyll *a* concentration fell progressively below that of the epilimnion until stratification ended in October.

The gross taxonomic composition of the phytoplankton

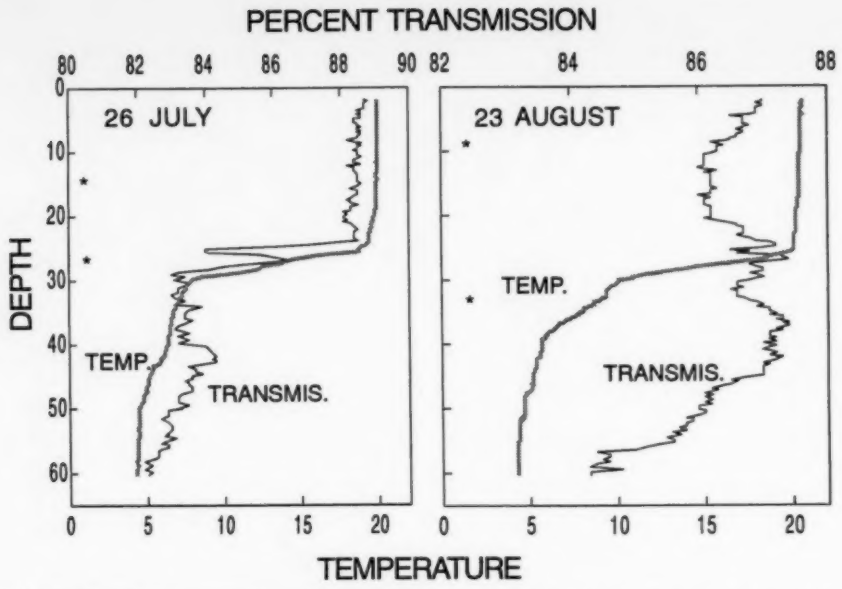

FIG. 1. Profiles of temperature (°C) and light transmission versus depth (m) on two dates in Colpoys Bay in 1991. Depths of sampling for surface and deep phytoplankton are indicated by stars.

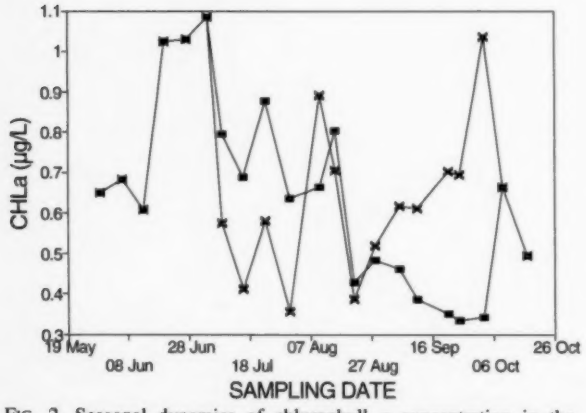

FIG. 2. Seasonal dynamics of chlorophyll *a* concentration in the epilimnion (crosses) and metalimnion (squares) of Colpoys Bay in 1991. Points shown are means for two depths within each layer. Superimposed symbols at the beginning and end of observation series denote isothermal conditions.

revealed a predominance of diatoms, flagellate chrysophyceans, and cryptomonads on all three experimental dates (Table 1).

The diatoms were mainly (47–93%) *Cyclotella* sp. throughout, but *Asterionella formosa* and *Fragilaria crotonensis* were important (>10%) in the metalimnetic populations on 24 July and 22 August, respectively. The chrysophyceans were heavily dominated (>70%) by small *Chromulina* sp. and *Ochromonas* sp., so their contribution to algal community biomass was almost certainly less than that of the diatoms. The cryptomonads were a roughly equal mix of *Rhodomonas* and *Cryptomonas* sp., except in the metalimnion of 26 July and 29 August, when

TABLE 1. Gross taxonomic composition of phytoplankton as a percentage of the total cell numbers on dates closely corresponding to those of the physiological experiments in 1991. EPI, epilimnetic samples; META, metalimnetic samples.

	24 July		22 Aug.		29 Aug.	
Taxon	EPI	META	EPI	META	EPI	META
Bacillariophyceae	27.4	33.5	19.7	44.3	40.2	31.7
Chrysophyceae	53.3	38.7	33.1	26.4	22.0	13.5
Cryptophyceae	11.2	10.6	28.6	16.4	19.5	41.3
Dinophyceae	0	2.3	4.1	2.7	1.8	1.0
Chlorophyceae	7.5	14.8	14.0	10.0	11.3	8.6
Cyanophycota	0.6	0	0.4	0	5.1	3.8

Rhodomonas sp. was >70% of the cryptomonad total. The chlorophytes were mainly small, unidentified forms.

Photosynthesis–Irradiance Relationships

The response of chlorophyll *a* specific photosynthesis to irradiance was adequately described by the chosen function (e.g., Fig. 3) in all cases. No significant value of the photoinhibition parameter, β, could be fitted in 12 of the 14 cases examined. Even when significant (surface populations at low temperature, and deep populations at high temperature, 26 July experiments), β was very small (0.000032 to 0.00019) and the degree of photoinhibition within the relevant range of natural irradiance was slight. All cases were therefore fitted without the photoinhibition term, for consistency, using only the parameters P_m^B and α (equation 2). In the two cases with significant photoinhibition, the resulting value of P_m^B was no more than 5% lower than P_s^B. The proportion of explained variation, R^2, ranged from 61 to 96% among the 14 cases, averaging 85%.

TABLE 2. *P–I* parameters for phytoplankton from Georgian Bay in 1991. LCL and UCL denote lower and upper 95% CI, respectively. Depth in m, temperature (Temp.) in °C, P_m^B in g C·g Chl a^{-1}·h^{-1}, α in [g C·g Chl a^{-1}·h^{-1}]·[μmol photons·m^{-2}·s^{-1}]$^{-1}$, and I_k in μmol photons·m^{-2}·s^{-1}.

Date	Depth	Temp.	P_m^B	LCL	UCL	α	LCL	UCL	I_k
26 July	15	13.5	1.60	1.46	1.75	0.019	0.014	0.025	83
		20.0	2.22	2.03	2.41	0.021	0.015	0.027	106
	27	13.5	1.20	1.08	1.32	0.029	0.022	0.036	41
		20.0	1.66	1.52	1.80	0.024	0.018	0.031	68
23 Aug.	10	11.0	0.203	0.018	0.224	0.006	0.004	0.008	33
		21.0	0.345	0.308	0.383	0.004	0.003	0.005	82
	35	11.0	0.221	0.188	0.252	0.006	0.003	0.009	36
		21.0	0.683	0.593	0.774	0.009	0.006	0.012	78
30 Aug.	7	11.0	0.760	0.676	0.844	0.015	0.010	0.021	51
		21.0	1.11	1.04	1.19	0.013	0.010	0.016	85
	21	11.0	0.792	0.682	0.901	0.014	0.008	0.020	57
		21.0	0.997	0.855	1.14	0.011	0.007	0.015	90
5 Oct.	5	6.0	0.46	0.42	0.50	0.018	0.013	0.024	26
		13.0	0.80	0.71	0.88	0.021	0.012	0.029	38

When compared at their respective temperatures, the surface populations always had a significantly higher P_m^B than the deep populations (i.e., their 95% CI's did not overlap, Table 2). When compared with identical temperatures, however, the surface and deep populations did not differ significantly in P_m^B on 30 August or at 11°C on 23 August. At identical temperatures, the surface populations had the higher P_m^B on 26 July but the deep population had the higher P_m^B on 23 August. Higher temperature during incubation for a given population was always associated with a higher P_m^B, and significantly so except for the deep populations of 30 August (Table 2).

Unlike P_m^B, α did not vary significantly between populations, whether compared at respective or at identical temperatures (Table 2). Temperature also had no significant influence on α for any given population in any of the seven comparisons that were made (Table 2).

The photoadaptation parameter, I_K, was consistently higher for surface than for deep populations when compared at their respective temperatures (Table 2). Using error propagation (Bevington 1969) to calculate 95% CI's for I_K from P_m^B and α, it was estimated that the populations so compared differed significantly on 26 July and 23 August, but not on 30 August. When compared at identical temperatures, a similar difference of I_K between populations was seen on 26 July but not 23 and 30 August, and none of the differences were significant. As was the case for P_m^B, temperature had a positive effect on I_K in each of the seven comparisons (Table 2).

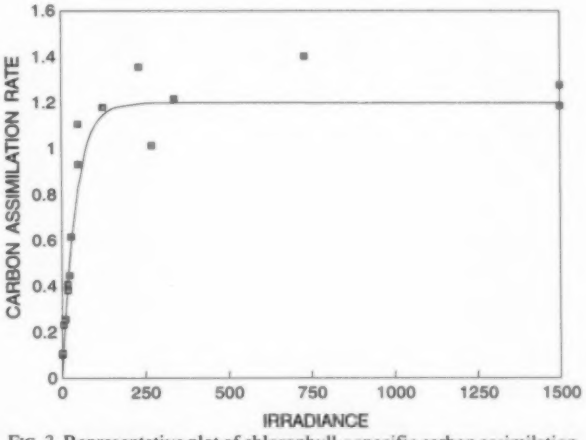

FIG. 3. Representative plot of chlorophyll *a* specific carbon assimilation (g C·g Chl a^{-1}·h^{-1}) versus photosynthetically active irradiance (μmol photons·m^{-2}·s^{-1}) for phytoplankton from Colpoys Bay (sampled 26 July 1991 from 27 m; incubated at 13.5°C). The line was fitted to equation 2 by nonlinear regression.

Photosynthate Allocation

The allocation of labelled photosynthate to individual end-products (as a percentage of the total) varied over a relatively small range (Table 3). ANOVA of the pooled data (with experiment as an additional classification variable) nonetheless indicated that the main treatments (depth, temperature, and light) had generally significant ($p < 0.05$) effects (Table 4). The only exceptions were the absence of a significant depth effect on allocation to polysaccharide and of a significant light effect on allocation of total lipid. Interactions between experiment and the main treatments (Exp.*, Table 4) were significant for protein and polysaccharide, indicating that the nature of the main effects varied among experiments. Interactions between depth and main treatments (Depth*, Table 4) were also significant for allocation to protein and lipid, evidence that surface and deep populations responded differently to the treatments. Allocation to LMW was very small and was not included in the analysis.

The main treatments, and their interactions with experiment and depth, explained most of the total variation in allocation to end-products (Table 4). Of the main treatments, depth was most important for protein and lipid, while temperature and light were of similar importance for polysaccharide (Table 4). Depth, and its interactions with other main treatments, explained 5–67% of the variation in allocation to the different end-products. Interexperiment variability, including interaction between experiment and main treatments, was also a major source of variation (Table 4).

The distribution of labelled photosynthate among lipid classes

TABLE 3. Allocation among photosynthetic end-products as a percentage of the total products. Values are means of duplicates.

Date	Depth	Temp.	Light	LMW	Polysaccharide	Protein	Lipid
26 July	15	13.5	30	4.1	57.5	33.5	8.5
			180	3.3	58.0	27.0	11.2
		20.0	30	4.0	56.0	35.5	7.3
			180	1.8	59.5	28.5	9.4
	27	13.5	30	3.7	55.0	30.5	11.0
			180	2.6	59.5	24.0	13.9
		20.0	30	2.6	58.5	29.0	9.1
			180	2.1	63.5	22.0	12.1
23 Aug.	10	11.0	30	6.4	63.5	23.5	6.5
			180	4.2	65.0	26.5	4.1
		21.0	30	4.5	60.5	29.5	6.7
			180	3.7	60.0	32.5	5.8
	35	11.0	30	6.3	63.0	21.5	9.3
			180	5.9	67.0	17.0	9.6
		21.0	30	4.8	62.5	21.5	10.8
			180	4.7	63.5	18.5	13.2
30 Aug.	7	11.0	30	6.3	63.5	20.2	9.3
			180	4.4	65.5	21.0	8.5
		21.0	30	4.6	59.0	34.0	7.5
			180	2.7	60.0	30.5	6.7
	21	11.0	30	5.5	63.0	21.5	10.1
			180	3.8	69.0	18.0	9.4
		21.0	30	3.2	57.5	26.0	12.5
			180	2.8	58.0	25.5	13.8
Mean				4.1	61.2	25.7	9.4
SD				1.5	3.9	5.5	2.9

TABLE 4. Contribution of main treatments and interactions to the explained variation (as a percentage of the total explained variation) for allocation classes and the total percentage of variation explained by the ANOVA model (R^2). Single asterisks denote interaction terms in the ANOVA model; double asterisks denote $p < 0.05$.

Factor	Protein	Polysaccharide	Lipid	Neutral lipid	Glycolipid	Phospholipid
Exp.	17.5**	36.0**	13.9**	16.5**	78.0**	53.7**
Depth	29.2**	2.3	55.9**	6.2**	0.8	6.8**
Temp.	15.4**	15.1**	0.2	13.4**	0.1	11.0**
Light	8.0**	13.3**	3.0**	36.8**	10.0**	6.1**
Exp.*	19.4**	28.8**	14.2	13.9**	8.6**	13.4**
Depth*	6.8**	3.0	7.4**	6.1**	0.5	1.8**
Depth* Exp.	3.7**	1.5	5.3	7.2**	2.0	7.1**
R^2	92.4	81.1	80.6	95.0	85.5	92.4

(as a percentage of the total for lipid) was more variable among treatments and experiments than the allocation among end-products (Table 5). ANOVA indicated that all the main treatment effects were significant for NL and PL, but only light was significant for AMPL (Table 4). Interactions between main treatments and both experiment and depth were significant for NL and PL, but only the experiment–light interaction was significant for AMPL.

The explained variation ranged from 85% (AMPL) to 95% (NL), with interexperiment variation (including interactions) again an important source of variation (Table 4). Of the main treatments, light was the single most important factor for NL (40% of explained variation), but none of the main treatments explained more than 12% of the variation in AMPL or PL. Depth and its interactions explained a relatively small part of the variation in allocation among lipid classes (Table 4).

The average patterns of allocation to end-products and lipid classes, and the biomass-specific rates of total photosynthesis, for the main treatments are summarized in Table 6. Total photosynthesis was substantially (and significantly) higher in higher-irradiance treatments. Most of the other main effects were small and/or nonsignificant, due to the interacting effects of main treatments and interexperiment variability. Depth effects on protein and lipid, temperature effects on protein and NL, and irradiance effects on NL were, however, significant at $p < 0.05$ (Table 6).

TABLE 5. Allocation among lipid classes as a percentage of the total lipid. Values are means of duplicates.

Date	Depth	Temp.	Light	NL	AMPL	PL
26 July	15	13.5	30	40.0	27.00	33.0
			180	50.5	25.50	24.0
		20.0	30	39.0	34.00	27.0
			180	55.5	23.00	21.5
	27	13.5	30	35.0	31.50	33.5
			180	57.5	19.50	23.0
		20.0	30	41.0	31.00	28.0
			180	60.0	23.50	16.5
23 Aug.	10	11.0	30	55.5	24.00	20.5
			180	59.0	19.50	21.5
		21.0	30	56.5	26.00	17.5
			180	65.0	18.00	17.0
	35	11.0	30	48.0	27.50	24.5
			180	50.5	14.00	35.5
		21.0	30	49.0	22.50	28.5
			180	59.5	10.00	30.5
30 Aug.	7	11.0	30	50.0	6.00	44.0
			180	54.0	6.50	39.5
		21.0	30	62.0	9.50	28.5
			180	56.5	8.50	35.0
	21	11.0	30	36.5	10.50	53.0
			180	47.0	12.00	41.0
		21.0	30	47.5	6.50	46.0
			180	63.5	11.00	25.5
Mean				51.6	16.7	29.8
SD				8.7	9.8	10.0

Discussion

Vertical heterogeneity of phytoplankton biomass and photosynthetic characteristics has been documented previously in a variety of freshwater and marine systems (e.g., Fee 1976, 1980; Cullen 1982; Platt et al. 1982; Gallegos et al. 1983) including the upper Great Lakes (Fahnenstiel and Glime 1983; Fahnenstiel and Scavia 1987a, 1987b; Fahnenstiel et al. 1989). The present results show that, for at least part of the stratified season, the phytoplankton in one location in nearshore Georgian Bay are also heterogeneous in their vertical distribution and physiological characteristics. A metalimnetic maximum of chlorophyll *a* concentration (Fig. 2) occurred for most of July, together with optical evidence (e.g., Fig. 1) for relatively high particle concentrations in the metalimnion. However, the metalimnetic maximum did not appear to persist after early August, even though thermal stratification continued. The disappearance of the metalimnetic maximum from Colpoys Bay in August contrasts with the reported situation in offshore Lakes Michigan and Superior, where the DCM persisted throughout thermal stratification (Fahnenstiel and Glime 1983; Fahnenstiel and Scavia 1987a).

When differences are reported between surface and deep phytoplankton populations, they usually are suggestive of a more shade-adapted condition in the deeper populations, as reflected in lower values of P_m^B and I_K (e.g., Platt et al. 1982; Fahnenstiel et al. 1989). We also observed such differences in Colpoys Bay (Table 2) if the surface and deep populations were compared at their respective in situ temperatures. However, it is recognized that such apparent differences in photoadaptive state may reflect the proximate effect of temperature differences on P_m^B and, consequently, on I_K (Platt et al. 1982; Fahnenstiel et al. 1989). Our experiments showed that proximate effects of temperature were indeed partly (July) or mainly (August) responsible for apparent differences in photoadaptive state. The development of sensitivity to photoinhibition may be a better indicator of differential photoadaptation between surface and deep phytoplankton (Platt et al. 1982; Gallegos et al. 1983), but no depth-related susceptibility to photoinhibition was found in the present study. Since there were also no significant differences in α between surface and deep populations, the evidence for true differential photoadaptation between surface and deep populations was limited to a modest difference in P_m^B in the one experiment conducted when the metalimnetic chlorophyll *a* maximum was presented.

It may seem anomalous that persistent thermal stratification, as observed in Colpoys Bay, is not associated with the development of a greater degree of differential photoadaptation between surface and deep populations. In laboratory cultures, microalgae can complete many photoadaptive changes on time scales less than their generation time (e.g., Post et al. 1984). In Colpoys Bay, thermal stratification persisted for at least 12 wk, or about 20 generations at a modest growth rate of 0.20/d. However, shade adaptation in the form of susceptibility to photoinhibition has been estimated to require 2–6 wk for full expression in arctic marine phytoplankton, while it can be supressed with exposures to higher irradiance of only 4–6 h in duration (Gallegos et al. 1983). Water motions within Colpoys Bay may be sufficient to preclude extensive photoadaptation if phytoplankton there behave similarly. The average irradiance relative to surface irradiance (I_a/I_0) as a function of mixing depth (Z_m) and light attenuation coefficient (k) may be calculated as

TABLE 6. Average physiological characteristics (with SD for $n = 24$) of phytoplankton from Georgian Bay with respect to each of the three primary variables (depth, temperature, and light) examined in three experiments during 1991. Double asterisks denote significant ($p < 0.05$) differences between pairs of means.

		Depth		Temp.		Light	
Attribute		Surface	Deep	Cold	Warm	Dim	Bright
P^B	Mean	0.61	0.75	0.61	0.75	0.38**	0.98**
	SD	0.52	0.38	0.38	0.52	0.12	0.46
LMW	Mean	4.2	4.0	4.7	3.5	4.7	3.5
	SD	1.4	1.6	1.6	1.1	1.4	0.5
Polysaccharide	Mean	60.7	61.7	62.4	59.9	59.9	62.4
	SD	3.3	4.4	4.4	2.9	3.6	3.9
Protein	Mean	28.5**	22.9**	23.7**	27.7**	27.2	24.2
	SD	5.3	4.3	5.0	5.4	5.6	5.2
Lipid	Mean	7.5**	11.3**	9.3	9.5	9.0	9.8
	SD	2.0	2.5	2.7	3.2	2.1	3.5
NL	Mean	53.6	49.6	48.6**	54.6**	46.7**	56.5**
	SD	7.9	9.2	8.0	8.6	8.6	5.6
AMPL	Mean	17.5	15.9	16.5	16.9	19.5	13.9
	SD	10.2	9.5	9.5	10.2	11.2	7.3
PL	Mean	27.4	32.1	32.7	26.7	32.8	27.5
	SD	8.9	10.6	10.6	8.6	10.8	8.7

$$(3)\quad \frac{I_a}{I_0} = \frac{1}{-kZ_m}[e^{-kZ_m} - 1]\,.$$

Equation 3, with appropriate choice of I_0, can be applied to calculate the average irradiance in a metalimnion of finite thickness, as well as in the epilimnion. The average I_a/I_0 for July and August was 0.39 for the epilimnion and 0.03 for the metalimnion, using a typical midsummer value for k as measured in 1991 (0.144/m). However, the range was at least 0.22–0.76 for the epilimnion and 0.004–0.17 for the metalimnion, based on observed differences in mixing depths over periods of days and weeks. Similar variations of mixing depth occur quite frequently in the nearshore of large lakes, as a consequence of surface water advection and internal seiche activity (Simons and Schertzer 1987). Furthermore, the time scale of 2–6 wk required for development of full sensitivity to photoinhibition (Gallegos et al. 1983) is comparable with the entire lifetime of the metalimnetic chlorophyll *a* maximum in Colpoys Bay (Fig. 2). The apparently limited residence time of phytoplankton in the metalimnion of Colpoys Bay, and probably other nearshore locations, may therefore set an additional limit to photoadaptive differences between surface and deep populations.

Stations further offshore will generally experience less influence from seiches and upwelling events. Offshore stations in Lakes Huron and Michigan are relatively stable in their stratification, and manifest clearer differences in photosynthetic behaviour between surface and deep populations in the limited number of comparisons made to date (Fahnenstiel et al. 1989). Where the effective boundaries between the relatively stable offshore regime and the more dynamic nearshore regime lie will depend on lake morphometry, orientation, and other factors. The boundary would appear to lie relatively far offshore in northern Lake Ontario, for example (Simons and Schertzer 1987), but may be much closer to shore in areas, such as western Georgian Bay, that are not so optimally oriented for coastal upwelling.

Patterns of intracellular photosynthate allocation may be more sensitive indicators of algal physiological condition than the rate of total photosynthesis (Morris 1981), and they did indeed indicate significant effects of depth and of interactions between depth and other main treatments. However, the magnitude of the effects was generally slight (Tables 3–5). The overall means for different depths were significantly different only for protein and lipid allocation (Table 6), and depth-related variation was less than 50% of the total explained variation for all allocation classes except lipid (67%). The allocation patterns thus appeared to reinforce the conclusion that surface and deep populations differed relatively little in their physiological condition. Fahnenstiel et al. (1989) similarly found relatively little difference in allocation patterns between surface and deep populations in three experiments in Lakes Michigan and Huron.

Interpretations of physiological condition must be tentative, however, because of the potentially confounding effects of multiple variables (especially light, temperature, and nutrients) and of differing species composition. We might, for example, expect that the lower irradiance and possibly higher nutrient availability in the metalimnion (Fahnenstiel et al. 1984) would favour greater allocation to protein in the deep than in the surface populations, especially relative to polysaccharide (Healey and Hendzel 1980; Morris 1981; Smith and Geider 1985). Instead, allocation to protein appeared roughly to obey the positive relationship with temperature reported by Lean et al. (1989) and was greatest in epilimnetic populations (Table 6). The influence of the differing species composition of the phytoplankton between surface and deep populations (Table 1) is very difficult to assess, partly for lack of sufficient studies of different taxonomic groups under controlled conditions, but also because the contribution of each taxon to community photosynthetic activity is not necessarily related to its abundance. At our crude level of taxonomic treatment, the differences between surface and deep populations were not great, but they may nonetheless have been significant. We did not attempt to enumerate photosynthetic picoplankton,

which may have contributed importantly (Pick 1991) and differentially to community photosynthesis in surface and deeper layers. In systems with stronger and more persistent DCM (e.g., Fahnenstiel and Glime 1983; Fahnenstiel and Scavia 1987a) the species composition generally differs strongly between surface and deep populations, and may pose an even greater challenge to interpretation.

Our experiments used light of the same, unnatural spectral quality for both surface and deep populations, even though the in situ spectra will vary between epilimnion and metalimnion. The effects of spectral quality on growth and primary production rates of phytoplankton are still poorly defined, and results are frequently inconsistent among investigators using different combinations of laboratory and field observations, as well as different light sources and experimental designs (e.g., Fee 1976, 1980; Pick and Cuhel 1986; Cuhel and Lean 1987; Rivkin 1989). One relatively consistent finding, however, is a tendency towards greater allocation of photosynthate to protein in blue and/or green spectra than in white light (e.g., Pick and Cuhel 1986; Rivkin 1989). We may therefore have underestimated the differences in allocation patterns between surface and deep populations that would be manifested in situ, under their differing incident spectra. Inasmuch as such differences are driven by the proximate effects of varying spectral quality, however, they would not alter our conclusion that the surface and deep populations failed to evolve a large degree of physiological differentiation, as evidenced by their similar performance under identical irradiance spectra.

Comparative data for Great Lakes phytoplankton are not abundant, but our results appeared to differ in several respects from reports for Lakes Huron and Michigan (Fahnenstiel et al. 1989) and Lake Ontario (Cuhel and Lean 1987; Lean et al. 1989), as well as a variety of other temperate marine and freshwater locations (e.g., Barlow 1982; Lean et al. 1989). Our estimates of allocation to protein and lipid were relatively low, while allocation to polysaccharide was relatively high. The protein to polysaccharide allocation ratio averaged less than 0.5 in our experiments, a value that frequently would suggest severe nutrient limitation (Healey and Hendzel 1980; Barlow 1982; Smith and Geider 1985). Variations in the protein to polysaccharide ratio may not always reflect nutrient limitation (Lean et al. 1989), but we also observed a relatively large allocation to NL (Table 4). Microalgae grown in culture usually allocate less than 10% of their lipid carbon to NL, which are expected to comprise mainly storage forms, unless they are nutrient limited or subject to some other stress that impairs cell reproduction (Parrish 1987; Lombardi and Wangersky 1991). In part, our results may reflect the relatively short duration of the experiments; longer incubations, especially if they include a dark period, often reveal a reallocation of photosynthate from polysaccharide to protein and, sometimes, lipid (Cuhel and Lean 1987; Lean et al. 1989; Smith and D'Souza 1993). This highlights the desirability of adopting standard protocols among investigators to facilitate comparison of results.

Patterns of lipid class synthesis confirmed the expectation that major lipid classes would respond differently to environmental variables. Allocation to NL was stimulated by higher irradiance (Tables 5 and 6), suggesting a role in relatively short-term energy storage in the phytoplankton of Colpoys Bay. Conversely, the AMPL, which include photosynthetic pigments and membrane components, tended to receive a greater share of the total lipid synthesized under lower irradiance (Table 5). Allocation to NL also increased with temperature, while allocation to phospholipids decreased. Improved predictions of lipid synthesis by phytoplankton in nature may therefore be possible if lipid is resolved into such classes of more uniform functional significance.

Our estimates of P_m^B, α, and I_K fell at the lower end of the range expected for such temperatures in phytoplankton from the Great Lakes (Fahnenstiel et al. 1989) and other temperate systems (e.g., Harrison and Platt 1986). This may again suggest that the phytoplankton of Colpoys Bay were nutrient limited or otherwise stressed to an unusual degree. Some oversight in our primary production protocol may have introduced the stress artificially, but our methods were comparable with those commonly used by others in oligotrophic systems. More data and even greater standardization of techniques are needed to determine whether the phytoplankton of Georgian Bay are indeed physiologically different from those elsewhere in the Great Lakes. It is interesting, however, that a quite different primary production protocol suggested previously that phytoplankton in Georgian Bay achieved significantly lower biomass-specific photosynthetic rates than in Lake Superior or Lake Huron (Munawar 1988).

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Patterns in Toxicity of Sublethal Mixtures of Metals and Organic Chemicals Determined by Microtox® and by DNA, RNA, and Protein Content of Fathead Minnows (*Pimephales promelas*)

Joanne L. Parrott[1] and John B. Sprague

Department of Zoology, University of Guelph, Guelph, ON N1G 2W1, Canada

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Combinations of copper (0–90 µg/L) and zinc (0–1200 µg/L) acted in an additive fashion when tested by Microtox®, decreasing the luminescence of the marine bacterium *Photobacterium phosphoreum* after a 30-min exposure. Acetophenone (0–18 mg/L) combined with diethylene glycol (0–40 g/L) had less-than-additive effects, as did acetophenone–zinc combinations. In contrast, less-than-additive action of copper (0–150 µg/L) and zinc (0–1500 µg/L) was apparent from DNA, RNA, and protein contents of newly hatched fathead minnows (*Pimephales promelas*) exposed for 4 d. The highest copper–zinc combinations caused dramatic decreases in DNA, RNA, and protein contents of fish to 35, 8, and 46% of control levels, respectively. Although most joint effects were less-than-additive, independent action and antagonism were observed for some combinations of toxicants, and the type of action usually changed as proportions of toxicants were altered. Isobole mapping of response surfaces gave a useful overall picture of joint action for all concentrations tested, was favoured as a method of illustrating the results, and provided the interpretations given above.

Des combinaisons de cuivre (0–90 µg/L) et de zinc (0–1 200 µg/L) agissaient de façon additive lors d'un test au Microtox®, faisant décroître la luminescence de la bactérie marine *Photobacterium phosphoreum* après une exposition de 30 min. L'acétophénone (0–18 mg/L) combiné au diéthylène-glycol (0–40 g/L) avait des effets moins qu'additifs, tout comme les combinaisons acétophénone–zinc. Par contre, l'action moins qu'additive du cuivre (0–150 µg/L) et du zinc (0–1 500 µg/L) était apparente dans l'ADN, l'ARN et les protéines chez des ménés tête-de-boule (*Pimephales promelas*) nouvellement éclos et exposés pendant 4 jours. Les combinaisons cuivre–zinc les plus élevées causaient des diminutions spectaculaires des teneurs en ADN, en ARN et en protéines, qui tombaient respectivement à 35, 8 et 46 % par rapport aux niveaux témoins. Bien que la plupart des effets combinés aient été moins qu'additifs, on a observé une action indépendante et de l'antagonisme pour certaines combinaisons de produits toxiques, et le type d'action changeait généralement quand on modifiait la proportion des toxiques. La cartographie par isoboles des surfaces de réponse fournissait une image globale utile de l'action combinée pour toutes les concentrations testées; elle a été retenue pour illustrer les résultats, et a fourni les interprétations ci-dessus.

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Profiles of the toxicity of many individual aquatic contaminants are now available and often include testing on several species and life stages and the modifying influence of pH, water hardness, and temperature. In contrast, there are very few studies on the sublethal toxicity of mixtures, although polluted waterbodies often have multiple toxicants. Lack of information on joint toxicity and absence of agreed methods of dealing with the topic represent a major gap in the practical aspects of controlling water pollution. The scarcity of studies on mixtures is partly because of the difficulty in carrying out long-term exposures at sublethal concentrations. The recent development of meaningful rapid tests of sublethal effect and microscale assays now makes it feasible to assess joint sublethal action for aquatic organisms. Recent thorough reviews (Broderius 1991, for lethal effects; Christensen and Chen 1991, for mathematical aspects) may signal increased interest in toxicity of mixtures.

The present work involved two rapid assays based on sublethal effect. The Microtox®[2] system measured a decrease in luminescence of the marine bacterium *Photobacterium phosphoreum* (Bulich et al. 1981). Assays with fathead minnows (*Pimephales promelas*) exposed newly hatched fish to toxicant for 96 h, after which DNA, RNA, and protein contents were measured as indicators of growth (Barron and Adelman 1984).

Two types of toxicants were chosen, the metals copper and zinc and the organic chemicals acetophenone (Ac) and diethylene glycol (Dg). Our selection was based on water solubility, chemical stability, and expected "similar" type of action (i.e., metal toxicity versus narcotic organic toxicity). The hypothesis was that additive joint toxicity would exist for the pairs of similar chemicals, while a combination of a metal with an organic

[1]Current address: Environment Canada, Rivers Research Branch, 867 Lakeshore Road, Burlington, ON L7R 4A6, Canada.

[2]Microtox is a trademark of Microbics Corporation, Carlsbad, Calif.

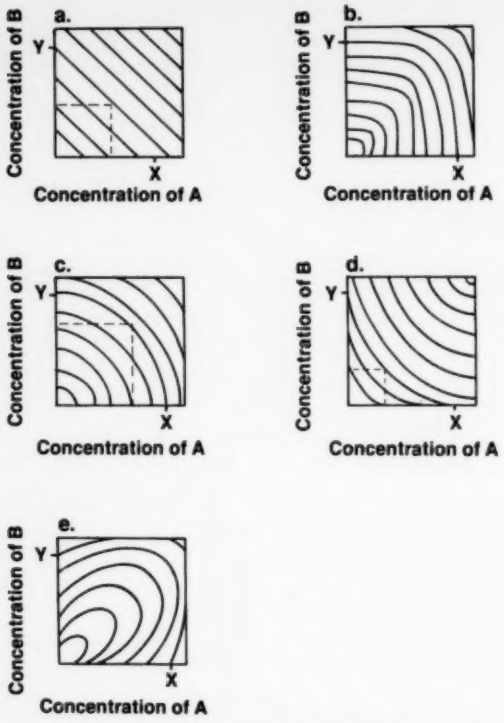

FIG. 1. Classifications of joint action based on isobole shape. (a) Additive joint action; (b) independent action; (c) less-than-additive action; (d) more-than-additive action; (e) antagonistic action.

chemical would cause less-than-additive effects because of the different modes of action. Microtox® was used for tests with metals and organic substances, while fish were exposed to a mixture of metals.

There are several ways to classify the actions of mixtures. The *concentration–addition model* (Anderson and Weber 1975) and *toxic unit concept* (Sprague and Ramsay 1965) assume that similarly acting toxicants, when mixed, will add together in causing an effect. To evaluate the joint action, the concentration of each toxicant in the mixture is divided by the concentration of that chemical which causes the specified effect when acting alone. The effect can be any one that is of biological interest, but the threshold of the median lethal concentration (incipient LC50) has often been used in experimental work. The sum of the fractions of LC50s for individual chemicals is the predicted effect of the mixture, which may be compared with an observed effect. Mixtures are classified as less-than-additive, additive, or more-than-additive when observed effects are less than, equal to, or greater than (respectively) the effects predicted by concentration addition.

In the *multiplicative model* (Rummens 1975; Nash 1981), the observed effects of a mixture's components acting singly (expressed as probabilities) are multiplied to predict joint action. For example, if given concentrations of either toxicant A alone or toxicant B alone caused growth to be 50% of the control level, the predicted effect would be 25% (0.5×0.5) of control growth when the same concentrations of A and B were mixed. The multiplicative model classifies mixtures that act according to the prediction as "additive," while those more or less toxic than predicted are termed "synergistic" or "antagonistic," respectively. (We do not agree with the extended definition of antagonistic, as indicated below.)

Isobole diagrams provide a visual display of joint action and were chosen to interpret the effects of toxicant pairs in this study. Isoboles are lines of equal effect, such as those indicating land elevation on topographic maps. The shapes of isoboles can illustrate toxicological joint action that is additive, more-than-additive, less-than-additive, independent, or antagonistic (see below). Because many combinations of concentrations are tested to derive a response surface, a picture is obtained of joint action at different proportions of the toxicants. This overall visual impression of joint action is an important advantage of isobole diagrams because joint action may vary at different proportions (EIFAC 1987).

The shape of isoboles has been used to judge the mode or modes of joint toxicity (Sprague 1970; Stebbing and Santiago-Fandino 1982; Ormrod et al. 1986; Broderius 1991; Christensen and Chen 1991). The joint action would be classified as *additive* if isoboles ran straight across from one toxicant axis to another at any angle. Figure 1a shows that *X* amount of toxicant A or *Y* amount of toxicant B caused a certain response. If 0.5 *X* (of toxicant A) plus 0.5 Y (of toxicant B) caused the same response (i.e., it fell on the same isobole), then additive action existed when the two toxicants were combined.

Independent action between toxicants would be indicated by a box-like isobole shape (Fig. 1b). A certain response would be caused by *X* amount of toxicant A, whether or not toxicant B were present, and vice versa. Horizontal and/or vertical isoboles would suggest that one or both toxicants were acting independently. *Less-than-additive* action would be diagnosed if isoboles curved away (convexly) from the toxicant axes, as in Fig. 1c. For example, if the combination of 0.7 *X* (toxicant A) and 0.7 *Y* (toxicant B) were necessary to produce the same effect as *X* or *Y* alone, the joint action would be less than expected using the additive model, and the term less-than-additive would be applied. A *more-than-additive* relationship between a pair of toxicants could be indicated by isoboles curving in (concavely) toward the toxicant axes (Fig. 1d). Under these conditions, only 0.3 *X* (toxicant A) plus 0.3 *Y* (toxicant B) might be required to produce the same effect as *X* or *Y* alone. *Antagonism* would describe action resulting in isoboles that were extremely convex or bulging (Fig. 1e). The toxicity expected from a given amount of toxicant A would be decreased by the presence of toxicant B, or vice versa, or both.

The objective of this work was to determine, for *sublethal* levels of toxicants, whether the hypothesized additive pattern prevailed for toxicants of similar action, with a less-than-additive pattern for substances of dissimilar action. The focus was on providing general interpretations that would be useful in practical applications, rather than exploring the theoretical aspects of joint action, or fitting very precise models to the observed patterns.

Materials and Methods

Microtox® was used to test a pair of metals, a pair of organic chemicals, and a organic–metal combination. A 5×5 matrix of concentrations resulted in 25 treatments for each pair of toxicants. Each 5×5 factorial was repeated three times. Standard procedures were used (Microbics 1988a, 1988b). Reconstituted bacteria were exposed at 15°C to osmotically adjusted (2%

NaCl) solutions of toxicant for 15 min for organic chemicals and 30 min for metals. Bacterial luminescence was then compared with preexposure luminescence, and effects were quantified as percent luminescence remaining (PLR), or for some purposes as the concentration causing 50% inhibition (the IC50).

For the Microtox® tests, we used metal concentrations of 0, 22.5, 45, 67.5, and 90 µg copper/L combined with 0, 300, 600, 900, and 1200 µg zinc/L (added as $CuSO_4 \cdot 5H_2O$ and $ZnSO_4 \cdot 7H_2O$). Concentrations of organic chemicals were 0, 4.5, 9, 13.5, and 18 mg Ac/L combined with 0, 10, 20, 30, and 40 g Dg/L. The organic–metal mixtures used the same five concentrations of acetophenone combined with 0, 338, 675, 1012, and 1350 µg zinc/L. Stock solutions were prepared 3h, and exposure solutions 5 min, before testing began. All solutions were made with hard, raw well water (hardness = 345 ± 20 mg $CaCO_3$/L (n = 134); alkalinity = 214 ± 10 mg $CaCO_3$/L (n = 132); mean ± SD).

The aim of the statistical analysis in both the Microtox® and fathead minnow experiments was to fit a response surface describing sublethal effects in terms of concentration. The full response surface model included individual toxicant concentrations, concentrations squared, combined toxicant concentrations, and replicate effects. Models were simplified by sequential dropping of nonsignificant terms followed by analysis of variance to determine whether the simplified model was a good fit (Petersen 1985; Ormrod et al. 1986). The simplest model which did not have statistically significant loss of fit ($P \leq 0.05$) compared with the full equation for the response surface was accepted as the fully reduced model and is given here.

Fathead Minnow Assay

The test with newly hatched fathead minnows was similar to that of Barron and Adelman (1984). Minnows less than 24 h old (20 fish per treatment) were exposed to metals for 4 d in 300-mL cylindrical plastic containers with mesh bottoms, contained within 500-mL glass beakers which held the 500 mL of test solution. The ratio of fish biomass to test water was about 0.1 g/L. The test water, glass, and plastic containers were replaced daily, and the mesh-bottomed containers facilitated the exchange. Fish were fed live brine shrimp twice a day. After 4 d, DNA, RNA, and protein contents were measured in a pooled sample of 10 fish from each treatment.

Groups of fish were exposed to copper at 0, 37.5, 75, 112.5, and 150 µg/L combined with zinc at 0, 375, 750, 1125, and 1500 µg/L. Concentrations probably decreased during 24 h, so results should not be used to judge effects caused by absolute concentrations. The nominal concentrations are considered adequate for purposes of this experiment since joint effects were always compared with the effect of each toxicant acting singly at the same time. Tests were conducted in hard, raw well water at 25.1 ± 0.3°C (mean ± SD; n = 273). The dissolved oxygen in test solutions before exposure was 97 ± 2% saturation (n = 35), dropping to 80 ± 3% (n = 100) after 24 h. Preexposure pH was 7.8 ± 0.24 (n = 35), rising to 8.2 ± 0.10 (n = 99) after 24 h.

Ten fish from each test condition were sonified in 1.8 mL of phosphate-buffered saline (PBS; KCl, 0.2 g/L; KH_2PO_4, 0.2 g/L; Na_2HPO_4, 1.15 g/L; NaCl, 8.0 g/L; $CaCl_2$, 0.1 g/L; $MgCl_2 \cdot 6H_2O$, 0.1 g/L; pH 7.5) and 0.75 mL of heparin (250 µg/mL; heparin from porcine intestinal mucosa; Sigma Chemical Company). Protein was measured later in frozen 400-µL portions of the fish homogenate. Protein content was determined spectrophotometrically by the Lowry method (Peterson 1977) using a protein assay kit (Sigma Chemical Company) and was expressed as micrograms per fish.

DNA and RNA were measured by the fluorescence of ethidium bromide, which binds to nucleic acids (Karsten and Wollenberger 1977). Newly prepared fish homogenate (200 µL) was placed in four iced microcentrifuge tubes, two containing 300 µL of PBS and two containing 250 µL of PBS and 50 µL of RNase (250 µg/mL; ribonuclease A from bovine pancreas; Sigma Chemical Company). Tubes containing RNase were incubated at 37°C for 30 min to degrade RNA. All tubes were then centrifuged at 10 000 rpm for 1 min and supernatants transferred to iced test tubes.

Fluorescence was measured with a Turner fluorometer (model 112) at an excitation wavelength of 365 nm and emission wavelength of 590 nm, comparing with a DNA standard of 5 µg calf thymus DNA/L (Sigma Chemical Company). Blanks contained 2.5 mL of 45 mg ethidium bromide/L in PBS. Three sequential additions of 100 µL of supernatant to each blank, with fluorescence measured, allowed calculation of a slope. The slope represented the combined amount of DNA and RNA for solutions without RNase; it represented DNA alone for samples that had been incubated with RNase. Four successive portions of 100 µL of standard DNA solution were then added to the same blanks, with fluorescence readings corrected for serial dilutions. The amounts of fish DNA plus RNA, and DNA alone, were calculated by comparing slopes with the slope of the DNA standard. RNA was calculated by subtraction, and division by 0.46 to allow for the lower affinity of single-stranded RNA for ethidium bromide (Karsten and Wollenberger 1977).

Results

Microtox®

Metals

The joint action of copper and zinc on bacterial luminescence was additive, judging by the straight lines between the copper and zinc axes of Fig. 2a, which shows PLR after 30 min (PLR 30) of exposure to metals.

The response surface in Fig. 2b shows the marked suppression of bacterial luminescence by copper–zinc mixtures. The highest fitted PLR 30 was 101% for the control, shown in the upper left-hand corner of the diagram.[3] Luminescence decreased along the zinc axis to 44.5% at the highest level of zinc alone (1200 µg/L) and decreased along the copper axis to 62.6% at the highest level of copper alone (90 µg/L). For copper and zinc combined at those highest concentrations, the predicted luminescence was –20.8% of its original value (upper right of Fig. 2b). In fitting the model, there was no limitation on negative numbers, although the most severe effect possible for an observed value with Microtox® would be a luminescence reading of zero. The simplified response surface (r^2 = 0.84) describing the effects of copper and zinc on bacterial luminescence was

$$\text{PLR } 30 = 101 - 0.430[\text{Cu}] - 0.0152[\text{Zn}] - 0.0000268[\text{Zn}]^2 - 0.000246[\text{Cu}][\text{Zn}].$$

Organic chemicals

Acetophenone (Ac) and diethylene glycol (Dg) had a less-than-additive effect on bacterial luminescence as shown by the curved isoboles of Fig. 3a. At low Dg concentrations (<20 g/L),

[3]The raw data are available in Parrott (1988), or may be obtained from the senior author.

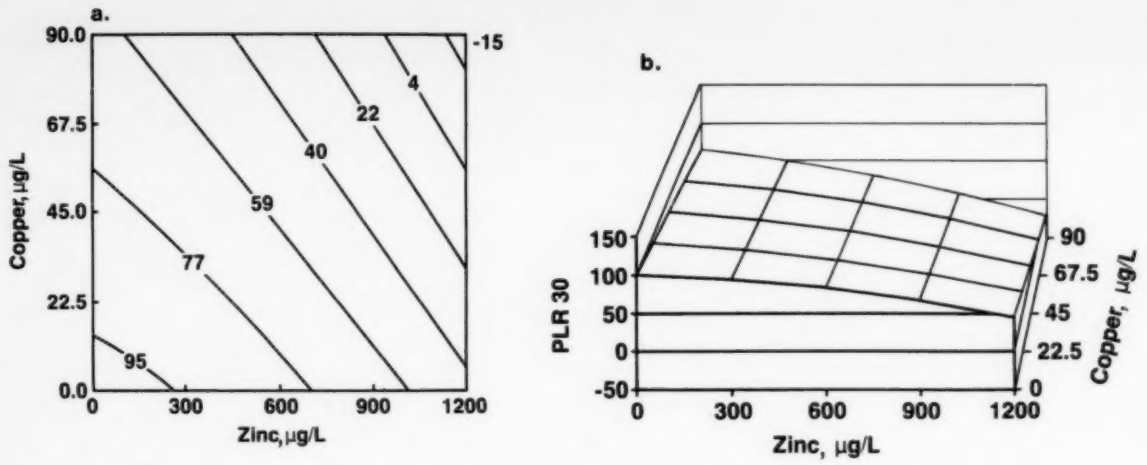

FIG. 2. (a) Isobole diagram for metals tested with Microtox®. Numbers on isoboles represent the predicted PLR after 30-min exposure to copper–zinc mixtures. (b) Response surface for metals tested with Microtox®.

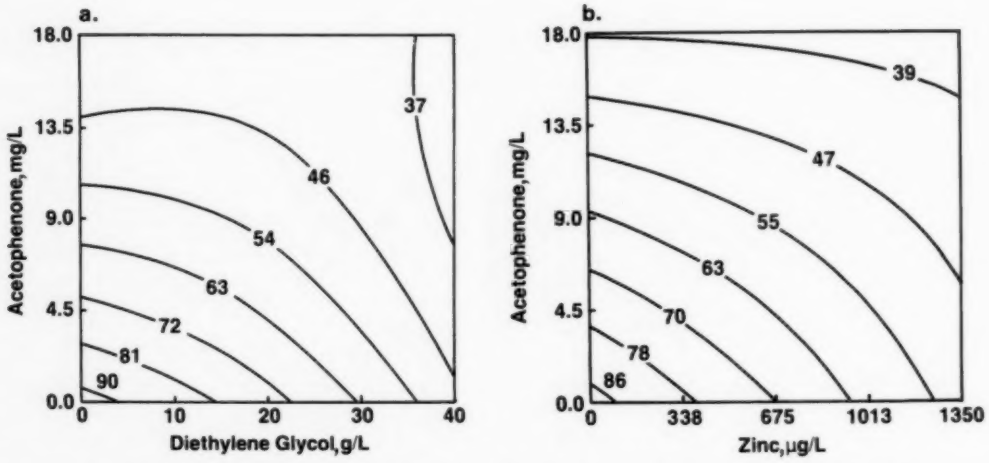

FIG. 3. Isobole diagrams for mixtures tested with Microtox®. Numbers on the isoboles represent the predicted PLR at the end of the exposures. (a) Ac and Dg tested with 15-min exposures (b) Ac and zinc tested with 30-min exposures.

some lines are nearly horizontal indicating independent action. The low levels of Dg apparently had little effect on the mixture's toxicity, with Ac mainly responsible for decreased luminescence. At higher levels of Dg (>20 g/L) the lines tend to be more slanted, showing some contribution of Dg to the mixture's toxicity. The response surface ($r^2 = 0.96$) describing the effects of Ac and Dg on bacterial luminescence was

$$\text{PLR } 15 = 92.7 - 4.37[\text{Ac}] + 0.0719[\text{Ac}]^2 - 0.643[\text{Dg}] - 0.0119[\text{Dg}]^2 + 0.0588[\text{Ac}][\text{Dg}].$$

Metals and organic chemicals

Zinc and acetophenone had somewhat less-than-additive effects on bacterial luminescence as indicated by the curved isoboles of Fig. 3b. Nearly horizontal lines at high Ac (>9 mg/L) and low zinc (<675 µg/L) suggest independent action, with Ac causing most of the reduction in PLR 30. Higher levels of zinc (>675 µg/L) did contribute to the mixture's toxic effects, since the lines slope down. This pattern was similar to that of Ac–Dg mixtures described above. The surface ($r^2 = 0.76$) describing the effects of Ac and zinc on percent luminescence remaining was

$$\text{PLR } 30 = 88.6 - 2.78[\text{Ac}] - 0.0272[\text{Zn}] + 0.00143[\text{Ac}][\text{Zn}].$$

Fish

DNA, RNA, and protein content of larval fathead minnows were reduced by 96-h exposure to the copper and zinc mixtures (Table 1). The highest concentrations of copper and zinc caused DNA and protein to decrease to about half, and RNA to decrease to less than one quarter, of control levels.

DNA content of fish was dramatically reduced by the copper

TABLE 1. Raw data for three replicates of fish exposed to metals. Measured DNA, RNA, and protein content of larval fathead minnows after 96-h exposure to copper and zinc combinations[a]. ND = not determined, as all 20 fish died.

Copper (μg/L)	Zinc (μg/L)	DNA (μg/fish)			RNA (μg/fish)			Protein (μg/fish)		
		Rep. 1	Rep. 2	Rep. 3	Rep. 1	Rep. 2	Rep. 3	Rep. 1	Rep. 2	Rep. 3
0	0	3.98	3.71	3.24	7.12	7.62	7.30	201	215	248
0	375	4.21	3.12	4.22	5.76	4.03	8.88	186	169	254
0	750	3.71	2.82	3.25	6.44	1.43	6.82	173	138	200
0	1130	2.33	2.46	2.92	1.00	2.68	3.42	110	133	175
0	1500	2.64	2.03	2.26	2.53	1.11	1.40	115	94	133
37.5	0	4.18	3.47	5.19	6.10	8.24	10.0	202	181	266
37.5	375	3.25	3.26	2.95	4.15	4.29	8.28	161	139	208
37.5	750	3.35	2.95	3.52	7.25	5.68	9.69	172	149	234
37.5	1130	3.78	2.83	2.62	0.74	4.31	4.04	138	139	173
37.5	1500	2.48	2.01	1.97	3.11	1.67	2.78	133	121	155
75.0	0	3.52	2.82	3.79	7.73	5.67	9.34	204	148	261
75.0	375	4.31	3.14	2.37	5.35	6.56	7.63	165	186	215
75.0	750	4.11	2.56	2.50	3.97	4.37	4.64	148	145	186
75.0	1130	2.89	2.50	0.95	3.15	2.70	1.36	143	113	92
75.0	1500	2.73	1.93	1.39	2.59	1.47	2.00	123	132	132
113	0	3.59	3.66	3.16	4.95	4.66	6.04	188	192	213
113	375	2.93	2.59	2.98	3.28	3.91	6.66	172	151	221
113	750	3.80	2.81	2.45	4.03	2.33	3.40	157	149	170
113	1130	2.56	2.55	1.57	1.56	3.67	2.06	115	126	129
113	1500	1.76	1.35	ND	1.36	2.51	ND	108	103	ND
150	0	3.53	2.65	3.00	5.99	3.19	4.44	133	151	190
150	375	2.88	2.99	2.09	3.09	2.24	2.86	125	129	161
150	750	3.27	2.78	0.79	3.00	3.49	0.75	184	105	87
150	1130	2.15	1.88	0.59	1.39	1.74	1.08	135	88	109
150	1500	2.45	1.43	ND	0.00	1.80	ND	114	106	ND

[a]These data were published (but with systematic errors in all DNA and RNA values) without the authors' knowledge in Ryan et al. (1992).

and zinc mixtures, with slightly less-than-additive effects indicated by somewhat curved isoboles in Fig. 4a. The fitted control level of DNA was 3.99 μg/fish (lower left of Fig. 4a). Exposure to 150 μg Cu/L reduced this to 3.05 μg (upper left of Fig. 4a), while zinc alone (1500 μg/L) reduced DNA to 2.34 μg/fish (lower right of Fig. 4a). Combined action of the same two levels of metal reduced the fitted DNA to 1.41 μg/fish (upper right of Fig. 4a and 4b). The response surface (Fig. 4b; $r^2 = 0.68$) describing the DNA content of fish (micrograms per fish) was

$$\text{DNA} = 3.99 - 0.00164[\text{Cu}] - 0.0000307[\text{Cu}]^2 - 0.00110[\text{Zn}].$$

RNA content of fish was decreased by combinations of copper and zinc in a generally less-than-additive manner as shown by curved isoboles in Fig. 4c. Fitted RNA of the control was 7.87 μg/fish, but this decreased to 3.95 μg/fish at the highest level of copper alone and to 1.48 μg/fish at the highest level of zinc alone. Together, the metals reduced expected RNA to 0.59 μg/fish, according to the response surface (upper right of Fig. 4c). Low levels of copper (<75 μg/L) showed no toxic contribution when combined with low levels of zinc (<750 μg/L) and were somewhat antagonistic to high zinc concentrations (>750 μg/L) as shown by bulging isoboles. Higher copper (>75 μg/L) did contribute to the mixture's toxicity, since the isoboles curve towards the copper axis. The response surface ($r^2 = 0.72$) describing the relationship between RNA (micrograms per fish) and the metals was

$$\text{RNA} = 7.87 + 0.00564[\text{Cu}] - 0.000212[\text{Cu}]^2 - 0.00426[\text{Zn}] + 0.0000135[\text{Cu}][\text{Zn}].$$

Protein content of fish was affected by copper and zinc mixtures in a less-than-additive manner, similar to the pattern shown in the RNA diagram. The response surface ($r^2 = 0.75$) for protein (micrograms per fish) and the concentrations of copper and zinc was

$$\text{PROT} = 222 - 0.0441[\text{Cu}] - 0.00244[\text{Cu}]^2 - 0.0695[\text{Zn}] + 0.000203[\text{Cu}][\text{Zn}].$$

Discussion

Copper and zinc were additive in their effect (bacterial luminescence) to less-than-additive (DNA, RNA, and protein of fathead minnows), judging by the isobole diagrams. Ac and Dg were less-than-additive in the Microtox® test, as were Ac–zinc combinations. The general expectation of additive or less-than-additive effect was borne out, as was the specific hypothesis that the pair of unlike substances would have less-than-additive effects on bacterial luminescence. However, the hypothesis of additive effects of like substances was not confirmed since less-than-additive results were frequent.

Evidence of independent action was the rule rather than the exception, in some part of the isobole diagrams for both of Microtox® and fish. Most independent action was at low concentrations of one chemical combined with high concentrations of the other, for example, with Ac and Dg (Fig. 3a). Similarly,

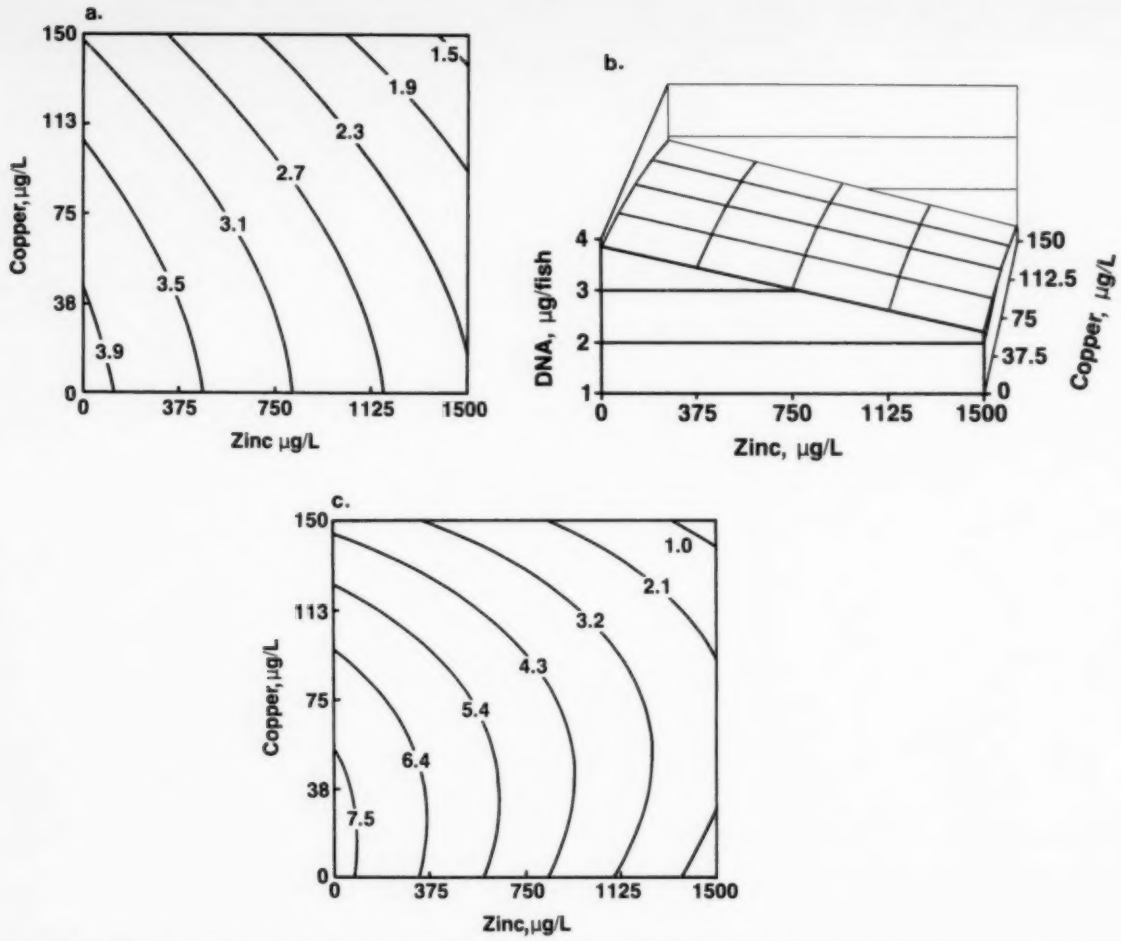

FIG. 4. DNA or RNA in larval fathead minnows after a 96-h exposure to copper–zinc mixtures. The numbers on isoboles represent the predicted DNA or RNA content in µg/fish. (a) Isobole diagram of DNA; (b) response surface describing the DNA content; (c) isobole diagram of RNA.

Ac was responsible for nearly all toxicity in mixtures with low concentrations of zinc (Fig. 3b). Independent action was also seen in certain effects on fish; low zinc concentrations reduced RNA (Fig. 4c) and protein, independent of low concentrations of copper. Antagonistic action was found only once; low copper concentrations had a small protective effect against high zinc concentrations for effects on RNA (Fig. 4c). No evidence of more-than-additive effects was seen.

A practical implication of these limited results for real-world situations would be that a simple assumption of additivity would provide a basis for adequate protection of surface waters receiving multiple contaminants. Although we found that effects of the mixtures were much greater than effects of single toxicants, most joint actions were less-than-additive.

There is general agreement with results in the literature for the additive to less-than-additive effects found in this study. Although tests of acute lethality to juvenile fish have often shown copper and zinc combinations to be additive (Lloyd 1961; Sprague and Ramsay 1965; Thompson et al. 1980) or more-than-additive (Anderson and Weber 1975; Lewis 1978; Anderson et al. 1979), Hermens et al. (1984) suggested that the potential for additivity decreased as concentrations changed from lethal to sublethal. They found additive lethal action for mixtures of up to 24 compounds, but less-than-additive sublethal inhibition of reproduction by *Daphnia magna*. Similarly, Spehar and Fiandt (1986) found additive effects in lethality of a six-metal mixture, but less-than-additivity for growth of fathead minnows. Using the Microtox® test, Hermens et al. (1985) found less-than-additive sublethal action of a mixture of organic chemicals, as we did, while Bois et al., (1986) interpreted the joint action of zinc and pentachlorophenol as additive.

There is also some evidence in the literature for a tendency toward independent action (such as that found in the present study) at high concentrations of one toxicant combined with low concentrations of another. Lethality tests with mixtures of three toxicants led to conclusions that an individual substance at 10% or less of its LC50 might not contribute to the toxicity of the mixture (Edwards and Brown 1967; Lloyd and Orr 1969). Eaton (1973) drew a similar conclusion from a study of the chronic effects of a tri-metal mixture on fathead minnows.

TABLE 2. Standard endpoints of sublethal toxicity derived from the equations for response surfaces. The endpoints shown for the two substances in each mixture represent the proportion of the two substances actually used in the exposures. IC50, inhibiting concentration for 50% reduction in light emission; TEC threshold-effect concentration; Ac, acetophenone; Dg, diethylene glycol.

	Cu (μg/L)	Zn (μg/L)	Ac (μg/L)	Dg (g/L)
Microtox® IC50 (test time)				
Cu, Zn separately (30 min)	120	1130	—	—
Ac, Dg separately (15 min)	—	—	12.2	38.7
Ac, Zn separately (30 min)	—	1420	13.9	—
In Cu–Zn mixture (30 min)	49.6	660	—	—
In Ac–Dg mixture (15 min)	—	—	9.70	21.6
In Ac–Zn mixture (30 min)	—	780	10.4	—

	Cu (μg/L)	Zn (μg/L)	Cu–Zn (μg/L) in mixtures
Fish TEC			
DNA measurements	>150	960	96/960
RNA measurements	130	960	59/590
Protein measurements	130	590	27/270

Effective Concentrations

Single endpoints of toxicity may be computed from the response equations of our data (Table 2) and compared with values in the literature. Our Microtox® IC50s for individual metals of 120 μg Cu/L and 1130 μg Zn/L are comparable with 30-min IC50s of 130 μg Cu/L and 1440 μg Zn/L obtained by Qureshi et al. (1984). Microbics (1982) reported a 15-min IC50 of 800 μg Cu/L, higher than ours, but a similar value of 900 μg Zn/L. Bois et al. (1986) showed a lower 30-min IC50 of 450 μg Zn/L, while Dutka and Kwan (1981) listed much higher 15-min IC50s of 3500 μg Cu/L and 3800 μg Zn/L. Our IC50s of 12 and 14 mg/L for Ac are comparable with the 15.5 mg/L reported by Kaiser and Palabrica (1991). Our IC50 of 39 g/L for Dg is of the same general magnitude as the 29 g/L reported by Hermens et al. (1985), but Microbics (1982) listed a smaller 5-min IC50 of 9 g/L.

The fathead minnow tests with DNA, RNA, and protein were less sensitive than tests in the literature which were based on whole-organism effects during life-cycle exposures. A standard endpoint would be the *threshold-effect concentration* (TEC), computed as the geometric mean of the lowest-observed-effect concentration (LOEC) and no-observed-effect concentration (NOEC). Our copper TECs of 130 to >150 μg/L (Table 2) were much higher than TECs of 22 and 28 μg/L for life-cycle tests with fathead minnows in hard water (Pickering et al. 1977). Similarly, our zinc TECs of 590–960 μg/L were much higher than a life-cycle TEC of about 73 μg/L for fathead minnows in hard water (Brungs 1969).

The 4-d LC50s for juvenile fathead minnows determined in our laboratory were 1150 μg Cu/L and 7360 μg Zn/L (Bowen 1990). In our sublethal tests, copper at 150 μg/L (about one eighth of its LC50) caused dramatic reductions in DNA, RNA, and protein to 76, 50, and 72% of the control, respectively. Similarly, zinc at 1500 μg/L (one fifth of its LC50) decreased these substances to 59, 19, and 53% of control values. Together, the highest copper–zinc concentrations caused dramatic decreases in DNA, RNA, and protein contents of fish to 35, 8, and 46% of control levels, respectively.

Other Models of Mixture Toxicity

Other models were applied to the data from this study (details in Parrott 1988). The concentration-addition model (Anderson and Weber 1975) generally gave the same classifications as the isobolic diagrams (additive and less-than-additive), but it presented simpler descriptions. For example, both models classified Ac–Dg and Ac–zinc mixtures as less-than-additive, but the concentration-addition analysis did not indicate that independent action was present at some combinations (Fig. 3). A greater difference was the more-than-additive action assigned by the concentration-addition model to copper–zinc mixtures tested by Microtox®, compared with the additive picture presented by isoboles. Similarly, concentration-addition categorized copper-zinc results for protein in fathead minnows as more-than-additive compared with the less-than-additive picture given by isoboles. The differences can be partly explained by the smoothing of the isobole response surfaces over peaks and ridges of the data. Most of the time that is beneficial since it removes minor variation and shows joint action more clearly. However, when "real" irregularities such as threshold values are removed or smoothed, the surface is not an accurate picture of joint action in that threshold area. The concentration-addition analyses were based entirely on calculations from TECs. Such threshold plateaus were smoothed in fitting surfaces for isoboles, so that loss of thresholds and asymptotic values is one of the drawbacks of the isobolic method.

The multiplicative model (Rummens 1975; Nash 1981) classified most of our findings as additive (Parrott 1988), which differed from the isobolic classification as additive to less-than-additive. For the most part, the contradictions resulted from greater error of regressions of the multiplicative model, based on only 15 data values compared with the 75 used to fit a response surface. Because of the greater error, the multiplicative model would require observed effects to show large departures from additivity in order to declare then significantly different from values predicted by additivity. The multiplicative model also has overly broad categories of joint action; for example, its classification "antagonism" includes three very distinct types of joint action, namely less-than-additive, independent action, and antagonism.

Isoboles provided useful visual images of the type(s) of joint action(s); it is important to assess variations in type of joint action for different proportions of toxicants. Many studies use only equitoxic mixtures, and would not detect patterns such as independent action at low levels of some toxicants combined with high levels of others. We acknowledge the criticism by Bois et al. (1986) that "isobolograms" are not suited for interpreting the action of mixtures but are uncertain of the validity of that criticism. Certainly isobolograms are being used for interpretation in authoritative reviews (Broderius 1991; Christensen and Chen 1991).

A disadvantage of isobolic representation is that it is limited to pairs of toxicants. If multitoxicant mixtures were of interest, isoboles for paired substances might be useful for initial studies to predict actions in the more complex mixtures, which could then be tested in experiments appropriately designed for minimal effort and cost. The response surfaces used to draw isobole diagrams also have poor threshold-modelling ability. If it is specifically desired to estimate thresholds of effect, then response surfaces should not be used.

A major difficulty in developing our experimental design for determining response surfaces and isobole diagrams was the necessity for all chosen concentrations in the 5×5 matrix to be "useful," i.e., none too high and none too low to provide useable responses. This constraint very much favoured a rapid test such as Microtox® over testing with fish. The rapid bacterial response allowed modification of toxicant concentrations, if necessary, before much effort was wasted. For future testing of mixtures, especially when using factorial designs, rapid assays such as Microtox® are recommended, at least for orientation. Some other investigators have already used Microtox® for studying mixture toxicity (Dutka and Kwan 1981; Hermens et al. 1985; Bois et al. 1986; Ribo and Rogers 1990).

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PERSPECTIVES

Emerging Role of Atmospheric Nitrogen Deposition in Coastal Eutrophication: Biogeochemical and Trophic Perspectives

Hans W. Paerl

Institute of Marine Sciences, University of North Carolina at Chapel Hill, Morehead City, NC 28557, USA

Paerl, H.W. 1993. Emerging role of atmospheric nitrogen deposition in coastal eutrophication: biogeochemical and trophic perspectives. Can. J. Fish. Aquat. Sci. 50: 2254–2269.

Approximately half of the world's oceanic primary production originates in coastal waters which are often nitrogen (N) limited and sensitive to externally supplied N. Among anthropogenically generated N sources, atmospheric deposition of N (ADN) (as wet- and dryfall) contributes 10–50% of the total external N load at present (20–100 mmol $N \cdot m^{-2} \cdot yr^{-1}$), and this will increase. At current levels, atmospheric N inputs lead to a significant enhancement of CO_2 fixation and chlorophyll *a* production. ADN may play a central role in mediating "new" production in coastal oceans downwind of emissions. Alterations in productivity, nutrient flux, and trophic changes have recently been documented from the Baltic and North seas, the Northwest Atlantic seaboard, and the Western Mediterranean. The recently noted "epidemic" of toxic and nuisance phytoplankton blooms is possibly due to accelerating nutrient loading inshore, especially from atmospheric sources. A need exists to spatially and temporally evaluate, quantify, and potentially manage this nutrient-production interaction on regional and larger synoptic scales. Aircraft- and satellite-based multiband ocean color remote sensing (SeaWiFS) will help characterize and link the spatial-temporal interactions of this (and other) "new" nutrient inputs with biogeochemical and trophic changes in coastal and offshore waters.

Environ la moitié de la production primaire des océans du monde provient des eaux côtières, qui sont souvent limitées en azote et sensibles à l'apport d'azote d'origine externe. Parmi les sources anthropiques d'azote, le dépôt d'origine atmosphérique de N (sous forme humide et sèche) représente 10 à 50 % de la charge totale de N d'origine externe à présent (20–100 mmol $N \cdot m^{-2} \cdot an^{-1}$), et cette proportion va s'accroître. Aux niveaux actuels, les apports atmosphériques de N conduisent à une augmentation significative de la fixation du CO_2 et de la production de chlorophylle *a*. L'azote d'origine atmosphérique peut jouer un rôle central de médiation de la « nouvelle » production dans les océans côtiers sous le vent des émissions. On a récemment étudié des modifications de la productivité, du flux de nutriants et des mécanismes trophiques dans la Baltique et la mer du Nord, sur la façade nord-ouest de l'Atlantique et dans l'ouest de la Méditerranée. La récente « épidémie » de proliférations phytoplanctoniques toxiques et nuisibles peut être due à une accélération de la charge en nutriants dans les eaux côtières, et particulièrement à l'apport atmosphérique. Il est nécessaire d'évaluer, de quantifier et d'essayer de gérer dans le temps et l'espace cette interaction de la production de nutriants à l'échelle régionale et à une échelle synoptique plus grande. La télédétection couleur multibande des eaux océaniques à partir d'aéronefs et de satellites (SeaWiFS) aidera à caractériser et à relier les interactions spatio-temporelles de cet apport de « nouveaux » nutriants (entre autres) avec des modifications biogéochimiques et trophiques dans les eaux littorales et pélagiques.

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Diverse biogeochemical and trophic studies have identified nitrogen (N) as being the nutrient that most frequently limits primary production in the marine environment (Dugdale 1967; Ryther and Dunstan 1971; Carpenter and Capone 1983). The rate at which externally supplied "new" N enters the oceans is a critical factor controlling primary production and associated C flux (Eppley and Peterson 1979). Accelerating primary production, or eutrophication, is particularly evident in estuarine and coastal waters impacted by enhanced "new" N inputs (Ryther and Dunstan 1971; Nixon 1986).

Traditionally, estuarine ecologists, oceanographers, and water quality managers have focused on terrestrial anthropogenically generated sources such as surface runoff, wastewater, and groundwater discharge as dominant "new" N inputs; however, recent nutrient source, flux, and budget assessments suggest that we need to expand our consideration of relevant man-made N inputs to include atmospheric deposition (Briblecombe and Stedman 1982; Buijsman et al. 1987; Duce et al. 1991). Man's steadily increasing consumption (combustion) of fossil fuels and accelerating industrial and agricultural discharges of N-containing gases, aerosols, and airborne particles are having a marked impact on the relative proportion as well as total amount of N loading derived from the atmosphere as wet- and

dryfall. Current estimates of the proportion of "new" N loading in estuarine and coastal waters originating from either direct or indirect (via runoff) atmospheric deposition range from 10 to over 50%, depending on location, hydrological regimes, and human activities (i.e., agriculture, industrial discharge, urban wastewater discharge, etc.) responsible for N loading. As we improve (reduce) land-based N loading, the proportions of terrestrial to atmospheric N loading appear to be changing, with the latter increasing. It therefore appears that quantitative and qualitative aspects of atmospheric N loading are growing in importance as we recognize and address issues of regional and global N flux pertaining to marine fertility, eutrophication, associated water quality, and fisheries resource management.

While not a specific focal point of this contribution, it is noted that atmospherically derived N loading in freshwater ecosystems, particularly those exhibiting N limitation, may also be of considerable importance in determining trophic and water quality conditions. In this regard, there is abundant evidence that lacustrine systems located in densely populated regions (Japan, Germany, United Kingdom, northeastern United States, and Canada) are currently impacted (and altered) by this "new" N source.

The following synthesis integrates current knowledge of coastal nutrient-production interactions and the potential role and impact of atmospheric deposition in phytoplankton nutrient (specifically C and N) assimilation, production, and eutrophication processes. Implications for biogeochemical cycling and trophic changes in affected waters are examined. In addition, the utility of technologies suitable for evaluating these interactions on appropriate spatial and temporal scales is explored.

Coastal Eutrophication: Biogeochemical and Trophic Significance

Coastal waters, including continental margins and shelves, continentally bound seas (i.e., Baltic and Mediterranean), and large estuarine systems, represent only 10% of the surface area of the world's oceans, yet account for approximately 40% of global oceanic primary production (Mackenzie et al. 1991; Mantoura et al. 1991; Wollast 1991) (Fig. 1). The relatively large share of oceanic production attributable to coastal waters is in part due to accelerating anthropogenic nutrient loading, leading to artificially enhanced rates of primary production or eutrophication (National Academy of Sciences/NRC 1969; Ryther and Dunstan 1971; Nixon 1986; Ambio 1990; Riegman 1990; Smetacek et al. 1991). Both magnitudes and proportions (relative to total oceanic primary production) of coastal primary production will increase as nutrient discharge associated with population growth and urban, industrial, and agricultural expansion steadily increases in coastal regions. As we enter the next century, nutrient discharge into coastal waters will probably accelerate (Halwagy et al. 1986; Smith et al. 1987; Brockmann et al. 1988; Ambio 1990; Mackenzie et al. 1991). Given this scenario, there is little doubt that the coastal oceans will continue to assume a growing share of global primary production.

The biogeochemical (e.g., C, N, P, and trace metals flux) and trophic (e.g., food web dynamics and eutrophication) implications of nutrient-enhanced primary production are poorly characterized, yet of considerable research and management concern from both regional and global ocean perspectives (D'Elia 1987; Brockman et al. 1988; Elmgren 1989). Of prime interest is the role accelerating coastal eutrophication will play in altering global CO_2 flux (i.e., the increasing role of coastal waters as a sink for atmospheric CO_2) and food web alterations (i.e., nuisance algal blooms), potentially detrimental to fisheries and recreational resources.

Both the amounts and sources of nutrients impacting coastal primary production are changing. While nutrient loading is generally increasing, specific nutrient sources and input/discharge trajectories are also changing, in part due to altered demography, changing patterns of human activities, emissions, and treatment/removal of nutrients impacting the coastal zone and beyond (Ryther and Dunstan 1971; Halwagy et al. 1986; Elmgren 1989; Duce 1991; Smetacek et al. 1991; Wollast 1991). Improved treatment of agricultural, municipal, and industrial wastes has, in certain instances, reduced nutrient loads from these sources, particularly in developed, industrialized regions (Halwagy et al. 1986; Fleischer et al. 1987). In contrast, atmospheric emissions, specifically N-containing compounds, have markedly increased over the past four decades in both industrialized and developing nations (Stensland 1980; Briblecombe and Stedman 1982; Galloway et al. 1984; Derwent and Nodop 1986; Placet et al. 1986; Rodhe and Rood 1986; Buijsman et al. 1987; GESAMP 1989; Duce et al. 1991). Presently, from 10 to over 50% of coastal N loading is attributable to atmospheric wet and dry deposition alone (Prado Fiedler 1990; Loye-Pilot et al. 1990; Duce 1991; Fisher and Oppenheimer 1991; Table 1), most of it entering by runoff, riverine/estuarine discharge, and direct deposition (Maybeck 1982; Duce 1991).

Enhanced atmospheric deposition of N (ADN) is receiving increasing scrutiny as a factor stimulating coastal primary production because (1) receiving waters are generally N limited and hence sensitive to N enrichment (Ryther and Dunstan 1971; Boynton et al. 1982; D'Elia et al. 1986; Nixon 1986; Howarth 1988), (2) wet and dry deposition at natural input (dilution) levels are known to stimulate primary production in N-limited Atlantic coastal and offshore waters (Paerl 1985; Paerl et al. 1990; Willey and Cahoon 1991), and (3) stable N isotope analyses of atmospheric N sources and receiving waters indicate that atmospheric N inputs (which have distinct isotopic "signatures"; see Table 4) form an important component of the "new" N pool supporting enhanced primary production in Atlantic coastal waters (Fogel and Paerl 1991; Paerl et al. 1993). Given recent increased frequencies and intensities of algal blooms (including "epidemics" of toxic cyanobacterial blooms in oligohaline estuaries and coastal phytoflagellate as well as toxic "red tide" dinoflagellate blooms) in waters often "downwind" of enhanced N deposition (Boalch 1987; Brockman et al. 1988; Paerl 1988; Smayda 1989, 1990; Paerl et al. 1990; Smetacek et al. 1991), ADN may play an important, yet thus far unassessed, role in supporting and enhancing "new" primary production (Fanning 1989; Legendre and Gosselin 1989; Paerl et al. 1990; Fisher and Oppenheimer 1991; Owens et al. 1992), associated CO_2 flux (Walsh et al. 1985; Paerl 1993), phytoplankton nuisance bloom potentials, and trophic (food web) changes in the coastal oceans (Paerl 1985, 1988; Paerl et al. 1990).

Nutrient–Production Interactions in Coastal Waters: Roles of Nitrogen and Other Nutrients

Until the 1960's, relatively little was known of nutrient-production interactions in the world's estuarine, coastal, and open-ocean waters (Dugdale 1967; Ryther and Dunstan 1971; Nixon 1986; Hecky and Kilham 1988). This was in stark contrast

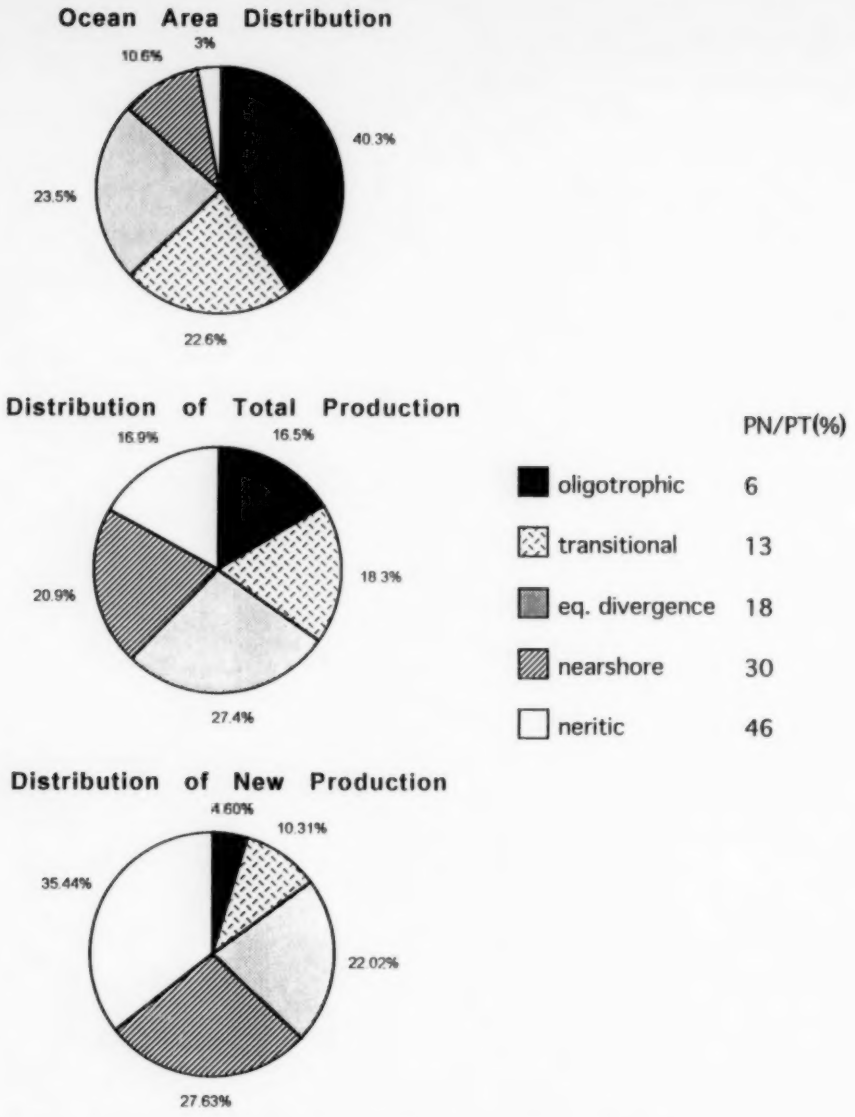

FIG. 1. Distributional graphs illustrating proportions of the world's ocean area, total primary production, and "new" primary production attributable to biogeographic originally defined by Eppley and Peterson (1979). Data are also derived from Eppley and Peterson (1979). Regions include (1) oligotrophic open-ocean waters, (2) transitional waters (offshore, but not necessarily oligotrophic open-ocean waters), (3) equatorial divergence waters, (4) nearshore waters, and (5) neritic (including estuarine) waters. The proportion of "new" to total ("new" plus regenerated) production is shown as PN/PT (%) for each region.

with limnological surveys which have clearly identified P and N (in that order) as key nutrients limiting freshwater primary production (Likens 1972; Vollenweider 1982; Hecky and Kilham 1988). Due to the ocean's vastness and associated logistical problems (e.g., adequate and appropriate research vessels, sampling apparatus, and onboard analytical requirements), efforts to identify and characterize limiting factors in the marine environment have lagged behind.

The pioneering work of Redfield and colleagues (Redfield 1958; Redfield et al. 1963) has been instrumental in identifying the stoichiometric relationship between phytoplankton elemental (C, H, N) content and concentrations of dissolved forms of nutrients in the sea. This approach, albeit an "indirect" means of prioritizing limiting nutrients, has been useful as a "rule of thumb" method for identifying potentially limiting nutrients. However, a direct method, capable of "letting the phytoplankton tell us," was needed to systematically test the myriad of potentially limiting nutrients in geographically and trophically diverse waters. To this end, nutrient-addition bioassays have identified several phytoplankton nutrients whose supply rates fall short of growth requirements in estuarine, coastal, and pelagic waters (Menzel et al. 1963; Dugdale 1967; Ryther and

TABLE 1. Summary of the estimated contributions of ADN to the total "new" N inputs in diverse estuarine, coastal, and open-ocean waters. When identified, the sources (wet (W) and/or dry deposition (D)) and chemical forms (inorganic (I) and/or organic (O)) of ADN are indicated.

Receiving waters	% of "new" N as ADN sources and forms	Reference(s)
Baltic Sea (proper)	>25% W + D, I	Rodhe and Rood 1986; Elmgren 1989; Anonymous 1990
Baltic Sea (Kiel Bight)	60% W + D, I	Prado-Fiedler 1990
North Sea (coastal)	20–40% W + D, I	GESAMP 1989; Anonymous 1990
Western Mediterranean Sea	10-60% W, I	Martin et al. 1989; Loye-Pilot et al. 1990
North Pacific Ocean (stratified surface waters)	40–70% W + D, I (NO_x)	Prospero and Savoie 1989
Sargasso Sea (surface waters)	~25% W, I	Duce 1986
Narragansett Bay, USA	5–20% W, I + O?	Nixon and Pilson 1993; NAPAP 1989
New York Bight, USA	~40% W, I + O?	NAPAP 1989
Chesapeake Bay, USA	20–40% W, I	NAPAP 1989; Fisher and Oppenheimer 1991
Neuse River – Pamlico Sound, N.C., USA	~27% W + D, I	Paerl et al. 1993
Atlantic Ocean coastal waters, N.C., USA	35–60% W + D, I	Paerl et al. 1993

Dunstan 1971; Nixon 1986).

Bioassay assessments of nutrient enrichment impacts on primary production have been conducted in a wide range of vessels (flasks, bottles, bags, larger scale mesocosms, and partitioned water bodies), incubated under natural (in situ) or laboratory conditions (Boynton et al. 1982; Grice and Reeve 1982; Nixon 1986; Paerl 1987; Oviatt et al. 1989; Paerl et al. 1990; Rudek et al. 1991). Nutrients are supplied on a single- or multiple-dose basis (batch culture approach) or on a flow-through chemostat basis, reflecting loading over time. The advantages and shortcomings of each approach have been subjects of extensive studies and debate (Grice and Reeve 1982; Hecky and Kilham 1988; Howarth 1988; Flynn 1989). Either natural phytoplankton assemblages or "indicator species" (easily cultured phytoplankton species reflecting a component of the natural community) are used as test organisms. A common approach is to add those nutrients suspected of controlling phytoplankton photosynthetic production (primary production), either individually or combined, over a range of concentrations or loading regimes. Growth responses are assessed by either CO_2 fixation, chlorophyll production, particulate organic C or dry weight measurements over time.

Advantages of bioassays over stoichiometric and other, more inductive approaches include (1) they can provide direct phytoplankton response information over a range of temporal and spatial scales, (2) depending on the growth parameter, initial inoculum, and environmental conditions, they can be highly sensitive indicators of nutrient limitation and nutrient-production interactions, (3) in the case of smaller microcosms (<10 L volume), they are easily deployed and readily replicated (Menzel et al. 1963; Ryther and Dunstan 1971; Vollenweider 1982; Paerl et al. 1990), and (4) in the case of larger mesocosms (100's to 1000's of litres), nutrient inputs, residence times, physical forcing (i.e., mixing and irradiance), and phytoplankton consumption (grazing) can be closely controlled (Harrison and Turpin 1982; D'Elia et al. 1986; Oviatt et al. 1989).

Disadvantages include (1) artificial constraints on nutrient transport and cycling by forced containment, (2) the well-known, but poorly understood, "bottle effect" induced by container surfaces which can alter microbial growth and community composition characteristics within the bioassay response time frame, (3) in the case of small vessels, radical alterations of physical (turbulence and irradiance) conditions may alter growth, biotic interactions, and community composition, (4) forced exhaustion of essential nutrients (not being examined) due to containment and lack of exchange with ambient waters — this is particularly problematic when air–water or sediment–water interactions are eliminated in closed vessels, and (5) uncertainty as to when and how to sample phytoplankton growth responses — what is a "realistic" time frame as to community or population nutrient limitation or response to nutrient enrichment with respect to nutrient assimilation

processes and phytoplankton generation times?

Despite arguable shortcomings and the potential for methodological and interpretive artifacts (Stockner and Antia 1976), bioassays deployed in geographically diverse estuarine and coastal waters suggest that phytoplankton N demand frequently exceeds availability in coastal waters and more saline segments of estuaries (Ryther and Dunstan 1971; Boynton et al. 1982; D'Elia et al. 1986; Nixon 1986, 1988; Howarth 1988; Oviatt et al. 1989; Graneli et al. 1990; Paerl et al. 1990; Fisher et al. 1992). In contrast, P limitation of phytoplankton growth is more commonly encountered in upstream, low-salinity regions of estuaries, reflecting its broad tendency to be a primary and colimiting nutrient in freshwater environments (Likens 1972; Vollenweider 1982; Hecky and Kilham 1988; Sommer 1989). P may also play a role as primary, secondary, or colimiting nutrient in mesohaline portions of estuaries (Taft 1976; Boynton et al 1982; D'Elia 1987; Harrison et al. 1990; Rudek et al. 1991), coastal seas (Baltic and Mediterranean), and embayments (Niemi 1979; Brockmann et al. 1988; Nelison and Stetels 1988; Anonymous 1990; de Jonge 1990; Riegman et al. 1992; Fourqurean et al. 1993; Schollhorn and Granéli 1993), often in response to periods of excessive N loading from runoff and direct atmospheric deposition inputs (Soderlund and Svensson 1976; Elmgren 1989; Karup 1989).

From a biogeochemical perspective, there are some fundamental differences in marine cycling of N and P, causing the former to be more "limiting" in terms of availability of biologically utilizable forms. An important difference is the fact that key N intermediates, including dinitrogen (N_2), ammonia (NH_3), and various nitrogen oxides (N_2O, NO, and NO_2), exist as gases and that these gases are readily exchanged between the hydrosphere and atmosphere (Delwiche 1981; Duce 1991). No analogous gaseous forms of P exist. P species exist as either dissolved solids (PO_4^{3-}) or organically and inorganically bound forms of P. P cycling is therefore confined to either the aqueous or solid phase, with no possibility of P loss to the atmosphere. Microbially mediated denitrification can be responsible for substantial losses of potentially available N (NO_3^-) to the atmosphere as N_2 (Berner and Berner 1987; Christensen et al. 1987; Seitzinger 1988). This loss can, to varying degrees, be compensated for by N_2 fixation, the process by which N_2 is biochemically converted to NH_3, another available form of N (Paerl et al. 1981; Capone and Carpenter 1982; Carpenter and Capone 1983). NH_3 (or ammonium, NH_4^+) can either be assimilated or microbially oxidized to NO_3^- (Ward 1986; Yoshida et al. 1989), which in turn can be denitrified (Goering et al. 1973; Hattori 1983; Codispoti and Christensen 1985). N budgets for estuaries and coastal waters indicate that N losses via denitrification generally exceed N gains by N_2 fixation (Rönner 1985; Christensen et al. 1987; Blackburn and Sørensen 1988; Howarth et al. 1988; Seitzinger 1988; Joye and Paerl 1993); the long-term ecosystem effect is chronic N deficiency. In contrast, estuarine and marine sediments and overlying waters are relatively (compared with N) enriched with P, which is efficiently recycled by microorganisms and infauna (Taft 1976). Faced with chronic net losses of N, but effective internal cycling of P, vast segments of these waters exhibit N limited conditions and are reliant on external N inputs for maintaining ecosystem fertility.

While N and to a lesser extent P are most often cited as being limiting in coastal and estuarine waters (Carpenter and Capone 1983; McCarthy and Carpenter 1983; Hecky and Kilham 1988; Howarth 1988; Oviatt et al. 1989), other nutrients may play important roles as either primary or secondary (synergistic) limiting factors. Si, Fe, and trace metals (Zn, Co, Mn, Cu, and Mo) are most often cited. In particular, Si availability can be an important determinant of phytoplankton community composition, since diatoms and silicoflagellates, numerically and functionally important constituents of coastal and estuarine phytoplankton communities, rely on adequate Si supply for meeting structural (e.g., frustules) and biochemical growth demands (Paasche 1980; Kilham and Kilham 1984; Schollhorn and Granéli 1993). Under conditions of high (excessive) N and P loading, Si may become limiting, leading to profound alterations of phytoplankton community structure and production (Officer and Ryther 1980; Schollhorn and Granéli 1993).

Fe has recently been proposed as playing a role as a limiting nutrient in open-ocean waters (Martin and Fitzwater 1988; Rueter et al. 1992). Interest in potential Fe limitation has intensified in regions of the Southern Ocean and the Eastern Pacific, which are characterized by relatively high biologically available N and P concentrations, yet low rates of primary production (Martin et al. 1990, 1991; Barber and Chavez 1991). It is felt that if no other nutrients were limiting, dissolved inorganic N and P species should be readily exhausted by phytoplankton assimilation. Martin et al. (1991) showed, by use of bioassays, that trace Fe additions led to enhanced phytoplankton production in Antarctic and Northeast Pacific waters. Rueter et al. (1992) have postulated a role for Fe limitation in the widespread planktonic N_2-fixing cyanobacteria *Trichodesmium* spp. H.W. Paerl et al. (unpublished data) have directly tested and verified Rueter et al.'s (1992) suggestion, using bioassays of natural and cultured *Trichodesmium* populations. Atmospheric dust may be a chief source of new Fe supporting primary production in these waters (Martin et al. 1991; Rueter et al. 1992).

Among coastal and estuarine waters, Fe supply has been hypothesized as playing a key nutritional role in the development and sustainance of coastal "red tide" dinoflagellate blooms (Martin 1975). Until now, little direct evidence has been generated to support this contention. In limnological and estuarine studies, Fe has frequently been identified as playing an important synergistic role, leading to additional growth enhancement when provided as a supplementary nutrient, most often in addition to N (Goldman 1972). By itself, however, Fe enrichment usually fails to stimulate phytoplankton production. Since Fe-containing enzymes and cofactors play key roles in N and C assimilation and transformations (NO_3^- reduction, N_2 fixation, and CO_2 fixation), the observed synergistic impact of Fe additions may be related to enhanced synthesis of these catalysts.

Other trace elements that have been mentioned as playing a potential role in controlling primary production include Zn, Mn, Co, and Mo (Huntsman and Sunda 1980; Bruland et al. 1991; Morel et al. 1991). In particular, the potential for Mo limitation has received attention, since like Fe, this metal is a cofactor in key N-transforming enzymes (nitrogenase and nitrate reductase). Because the most readily available ionic form of Mo, molybdate (MoO_4^{2-}), is a structural analog of the abundant anion, sulfate (SO_4^{2-}), in seawater (28 mM), Mo availability may be constrained through competitive inhibition (in the uptake process) by SO_4^{2-} in coastal waters (Howarth and Cole 1985). However, cellular requirements for Mo are exceedingly small, and effective storage of Mo has been demonstrated among N_2-fixing organisms having an absolute requirement for this trace metal (Ter Steeg et al. 1986). In addition, dissolved MoO_4^{2-} concentrations are highly conserved in near-surface euphotic waters, indicating

a lack of localized depletion (Collier 1985). Lastly, a variety of nutrient-addition bioassays in oxic and anoxic waters reveal a consistent absence of Mo (as well as other trace metals, including Zn, Mn, Co, and B) limitation among either N_2-fixing or NO_3-assimilating planktonic communities (Paerl et al. 1987, 1993; Paulsen et al. 1991). It is therefore concluded that in the case of Mo, no large-scale role as a limiting nutrient appears to exist.

Enhanced Nutrient Loading in Relation to Coastal Eutrophication: What is the Evidence?

The functional relationships between enhanced nutrient loading and eutrophication are well documented among heavily impacted inland waters and have become the subject of intense management efforts aimed at arresting this phenomenon. Prior to World War II, analogous estuarine and coastal examples were virtually nonexistent, in part because these systems were not studied intensively and their sheer vastness and volume effectively diluted nutrient and other pollutant inputs. Human encroachment, in the form of coastal (and upstream) population expansion, increased domestic and industrial wastewater inputs, agricultural growth, accompanied by conversion of forest to grazing and crop land, increased soil and sediment loss, as well as accelerating use (and loss) of fertilizers, has jointly contributed to an alarming rise in nutrient loading in the coastal zone and beyond (Correll and Ford 1982; Fleischer et al. 1987; Brockmann et al. 1988; Billen et al. 1991; Smetacek et al. 1991). Estuaries and coastal waters previously considered pristine and unaffected by eutrophication have been dramatically altered by excessive nutrient, sediment, and toxin loading in the post World War II era (Stienen 1986; Boalch 1987; Nelison and Stetels 1988; Elmgren 1989; de Jonge 1990). Some striking examples include the Scheldt, Rhine, Humber, Elbe, and Po estuaries in Europe and Chesapeake, Galveston, and San Francisco bays in North America.

Coastal waters and seas have more recently revealed troubling symptoms of eutrophication, in the form of distinct shifts in phytoplankton community composition (generally away from desirable forms such as diatoms and flagellates towards undesirable groups such as toxic dinoflagellates, prymnesiophytes, and cyanobacteria), resultant nuisance blooms, associated bottom water anoxia, fish kills, and radical shifts in invertebrate grazer and fish communities (Cadée and Hegeman 1986; Lancelot et al. 1987; Brockmann et al. 1988; Paerl 1988; Cosper et al. 1989; Skjoldal and Dundas 1989; Smayda 1989, 1990; Riegman et al. 1992).

Particularly alarming is an expanding list of "first time ever bloom events" (Table 2). The past two decades have seen particularly notorious events, some of which are elaborated on here. Examples include a disastrous "brown tide" (*Aurecoccus anophagefferens*) in U.S. Northeast coastal waters, which has devastated shellfish and other aquaculture industries (Cosper et al. 1989), and increasing frequencies and intensities of toxic "red tide" events along the U.S.-Canadian Northwest, U.S. Gulf, Southeast, and Northeast, U.S.-Canadian Atlantic, and Western European coasts (Anderson 1989; Smayda 1989). In this regard, a "first ever" outbreak of the Florida red tide dinoflagellate *Ptychodiscus brevis* (*Gymnodinium breve*) was reported as far north as North Carolina in the fall–winter of 1987–88 (Tester et al. 1989); this bloom led to approximately $40 million losses in fishing and recreational income in North Carolina alone.

Expanding blooms of the toxic dinoflagellate *Alexandrium* along the New England Atlantic coast (Anderson 1989) and increasing frequencies and geographic distributions of the dinoflagellate *Gyrodinium aureolum* in the North Sea and along the Norwegian coast (Braarud and Heindal 1970; Tangen 1983) represent a growing threat to coastal fisheries and aquaculture interests. Expansion of the toxic (to shellfish) dinoflagellate *Gymnodinium catenadum* in coastal regions worldwide is particularly problematic in mariculture (fish and shellfish) regions of Japan, Spain, and Australia (Smayda 1989). Toxic dinoflagellates (*Pyrodinium bahamense*) are similarly expanding in Indo-Pacific coastal (and offshore) regions (McLean 1984). An alarming rate of increase in dinoflagellate red tides since the mid-1970's has characterized Hong Kong Harbor (Lam and Ho 1989).

Toxic diatom blooms (*Nitzchia pungens*, the causative agent of amnesic shellfish poisoning (ASP) attributable to domoic acid production) appear to be increasing in frequency along the U.S.–Canadian Pacific and U.S. Northeast and Canadian Maritime Atlantic coasts (Bates et al. 1989). Expanding prymnesiophyte (*Phaeocystis* spp.) blooms have been observed along continental margins of the North Atlantic and in the North and Baltic seas (most notable along the Norwegian, U.K., and Dutch coasts) (Lancelot et al. 1987; Riegman et al. 1992).

Recently documented toxic blooms of the flagellated prymnesiophyte *Chrysochromulina polylepsis* in the low-salinity Western Baltic and higher salinity Kattegat–Skagerrak regions joining the Baltic and North seas are highly suggestive of large-scale nutrient-enhanced eutrophication (Skjoldal and Dundas 1989). During a particularly virulent bloom, this "killer alga" wreaked havoc with the economically important salmon-rearing farms along the Norwegian Baltic – North Sea coasts; mass mortality of pelagic fish and seals was reported in affected waters (Underdal et al. 1989). Changing N:P ratios, reflecting enhanced N loading and locally reduced P loading (attributable to improved wastewater treatment and reduction of P discharges) as well as increased N:Si and P:Si ratios, may be responsible for the appearance and exceptional toxicity of this and other bloom formers (Skjoldal and Dundas 1989; Smayda 1989). Additional prymnesiophyte blooms, most notably the gelatinous colony former *Corymbellus aureus*, are becoming a common feature of the North Sea (first seen in 1983) (Gieskes and Kraay 1986).

Intensifying blooms of the newly discovered chrysophyte silicoflagellate *Dictyocha speculum* have also been reported (Jochem and Babenerd 1989); what makes this bloom former unique and of considerable interest is its absence of a silicious skeleton. It has been suggested that this structural modification is the direct result of increasing N:Si ratios, attributable to excessive N loading in the Baltic Sea (Kiel Bight) where this phytoplankter resides (Jochem and Babernd 1989), an ominous sign of increasing eutrophication in this region.

Extensive blooms of the heterocystous cyanobacteria *Nodularia spumigena* and *Aphanizomenon flos-aquae* have plagued water quality (as causative agents for associated bottom water anoxia and hypoxia) throughout nutrient-enriched regions of the Baltic Sea (Horstmann 1975).

A comprehensive compendium of recent novel phytoplankton blooms and their geographic expansion can be obtained by consulting Cosper et al. (1989), Smayda (1989), and Smetacek et al. (1991).

A consistent and troubling theme that pervades virtually all these events is accelerating anthropogenic N, and in some cases P loading (e.g., N_2-fixing cyanobacterial blooms in the Baltic),

TABLE 2. Recently reported (since mid-1960s) accelerating (increasing in frequency and magnitude) as well as "first ever" major nuisance phytoplankton bloom events in coastal and estuarine waters currently impacted by accelerating atmospheric deposition of nutrients (chiefly N). In the context of water quality problems, nuisance taxa include those responsible for (1) potentially problematic physical/chemical alterations (e.g., bottom water hypoxia and anoxia, excessive biological oxygen demand leading to the appearance of sulfide in hypolimnetic waters, significant discoloration or turbidity changes in affected waters leading to altered light transmittance, and odor and taste problems) and (2) negative biotic impacts including (a) toxicity, (b) altered edibility or food value, and (c) parasitism and disease-inducing properties.

Bloom organism (major taxonomic group)	Impact	Date/location	Reference(s)
Gyrodinium aureolum (dinoflagellate)	2a	1966, North Sea	Braarud and Heimdal 1970
Alexandrium tamarense (dinoflagellate)	2a	1968, North Sea	Smayda 1989
Nodularia spumigena (cyanobacterium)	1, 2b	1969 on, Baltic Sea	Horstmann 1975; Niemi 1979
Prorocentrum minimum (dinoflagellate)	1	1976, North Sea	Kat 1988
Phaeocystis pouchetii (flagellate)	1	1977 on, North Sea	Gieskes and Kraay 1977; Boalch 1987
Gyrodimium aureolum (dinoflagellate)	1, 2a, 2b	1978 on, Hong Kong	Lam and Ho 1989
Aphanizomenon flos-aquae (cyanobacterium)	1, 2b	1978 on, Baltic Sea	Kononen and Niemi 1984
Corymbellus aureus (colonial microflagellate)	1, 2a, 2b	1983, North Sea	Gieskes and Kraay 1986
Gymnodinium catenadum (dinoflagellate)	2a, 2c	1980s on, Pacific: Japan; Mediterranean-Spain	Hallegraeff 1993
Alexandrium tamarense (dinoflagellate)	2a, 2b	1980s on, U.S. Northeast Atlantic	Anderson 1989
Dictyocha speculum (silicoflagellate)	2b	1983 on, Western Baltic	Jochem and Babenerd 1989
Aureococcus anophagefferens (chrysophyte)	1, 2a, 2b	1985 on, U.S. mid-Atlantic and Northeastern coastal waters	Cosper et al. 1989
Ptychodiscus brevis (*Gymnodinium breve*) (dinoflagellate)	1, 2a, 2b	1987–88, U.S. North Carolina Atlantic Coast	Tester et al. 1989
Chrysochromulina polylepsis (microflagellate)	1, 2a, 2b, 2c	1988, Western Baltic	Asknes et al. 1989

to affected waters (Niemi 1979; Billen et al. 1985; Stienen 1986; D'Elia 1987; Fleischer et al. 1987; Brockmann et al. 1988; Elmgren 1989; Riegman 1990; Smetacek et al. 1991). In many of the systems discussed above, N loading has doubled to tripled over the past three to four decades; P loading has increased in somewhat smaller, yet significant, increments during this time frame (Smayda 1989; Anonymous 1990; Smetacek et al. 1991). Increased nutrient loading has not only led to enhanced symptoms of eutrophication; ionic ratios of potentially limiting nutrients have also been altered. In particular, relatively high rates of anthropogenic N loading in coastal and estuarine waters have led to increases in N:Si ratios (Smayda 1989; Schollhorn and Granéli 1993).

Enhanced P loading has also been responsible for steadily increasing P:Si ratios. One consequence of this nutrient ratio modification is a noticeable and steady trophic shift away from high-Si-requiring diatoms and silicoflagellates, in favor of potential nuisance taxa such as prymnesiophytes and dinoflagellates and, in more brackish and freshwater environments, bloom-forming cyanobacteria (Paerl 1988; Jochem and Babenerd 1989; Smayda 1989). Current accelerating rates of anthropogenic N loading are viewed with concern because most receiving waters are N limited and hence susceptible to further eutrophication by this nutrient. Compounding this concern is the fact that P input constraints, including improved P removal from wastewater, P detergent bans, reductions in P loss from soils, and better-managed P fertilizer applications, have been more aggressively pursued (largely because they are more cost effective, easily implemented, and technologically feasible) than N input constraints, which are often diffuse (e.g., non-point sources), and expensive to deal with.

Dynamics of N Loading in Coastal Waters: Role of Atmospheric Deposition

In terms of anthropogenic generation and discharge of nitrogenous wastes, we have traditionally focused on familiar, tangible sources, such as wastewater (sewage and industrial) and runoff from fertilized and otherwise disturbed agricultural, urban, and rural regions (Soderlund and Svensson 1976; D'Elia

1987; Fleischer et al. 1987; Nelison and Stetels 1988; Elmgren 1989; de Jonge 1990). An additional, hitherto poorly assessed source of anthropogenic N loading is the atmosphere (Menzel and Spaeth 1962; Rodhe et al. 1980; Galloway et al. 1984; Duce 1991; Duce et al. 1991; Fisher and Oppenheimer 1991). Specifically, the combustion of fossil fuels and discharge of agricultural and industrial N-containing compounds into the atmosphere (as gases, aerosols, and fine particles) constitute a highly significant, and growing, percentage of total N loading into estuarine and coastal waters (Rodhe et al. 1980; Stensland 1980; Altshuller 1984; Derwent and Nodop 1986; Duce 1986; Rodhe and Rood 1986; Luke and Dickerson 1987). Current estimates of the percentage of total (natural plus anthropogenic) N loading attributable to atmospheric sources range from approximately 10% in waters adjacent to sparsely populated nonindustrialized regions to well over 50% in waters "downwind" of urbanized, industrialized, and intensively farmed regions (Correll and Ford 1982; Martin et al. 1989; Loye-Pilot et al. 1990; Prado-Fiedler 1990; Duce 1991; Duce et al. 1991; Fisher and Oppenheimer 1991; Paerl et al. 1993). Major European, North American, and Asian estuaries and coastal ecosystems, all exhibiting symptoms of eutrophication and phytoplankton bloom "epidemics," are currently impacted by atmospheric N deposition. Included are the North and Baltic seas (Fig. 2A), associated tributaries and estuaries (Anonymous 1990; Smetacek et al. 1991), North American estuaries, including Chesapeake Bay (D'Elia 1987; Fisher and Oppenheimer 1991) (Fig. 2B) and North Carolina's Albemarle–Pamlico Sound System (Paerl et al. 1990, 1993), the Western Mediterranean Sea (Martin et al. 1989; Loye-Pilot et al. 1990), Long Island Sound and North American coastal waters stretching from the Northwest (Puget Sound and Strait of Georgia), and Gulf Coast, mid-Atlantic, and New England northward along the Canadian Maritime Provinces (Anderson 1989; Smayda 1989). In these and other impacted regions, annual atmospheric N deposition rates range from 20 to 100 $mmol \cdot m^{-2} \cdot yr^{-1}$ and are increasing, largely a reflection of human population growth and urbanization, industrialization, and agricultural expansion worldwide (Table 3). In Western Europe and North America, anthropogenic ADN has increased from ~50 to over 200% since World War II (Rodhe et al. 1980; Stensland 1980; Briblecombe and Stedman 1982). Even remote oceanic waters of the mid-Pacific, Atlantic, and Indian oceans are now impacted by detectable anthropogenic atmospheric N inputs (Jickells et al. 1982; Prospero and Savoie 1989; Duce et al. 1991; Owens et al. 1992; Table 3).

The chemical composition of atmospheric N deposition is such that a large proportion of it should be readily utilized by phytoplankton (as well as attached microalgae and higher plants) in receiving waters. Fossil fuel combustion, a major source of ADN worldwide (Briblecombe and Stedman 1982; Logan 1983; Altshuller 1984; Levy and Moxim 1987; Duce et al. 1991), generates large amounts of nitrogen oxides (NO_x) (Likens et al. 1974). When dissolved in water, either nitrite (NO_2^-) or nitrate (NO_3^-) is the resultant anion; both serve as excellent plant nutrients. Agricultural (atmospheric) discharges are dominated by gaseous or ionized ammonia (NH_3 and NH_4^+) and NO_x (Duce 1986; Buijsman et al. 1987), which are available to plants. Both NO_x and NH_3/NH_4^+ are found in wet and dry (aerosol or particle associated) deposition. In either case, these soluble N species are rapidly liberated in receiving waters.

Organic nitrogen (ON) is also thought to be a quantitatively important fraction of ADN (Correl and Ford 1982; Mopper and

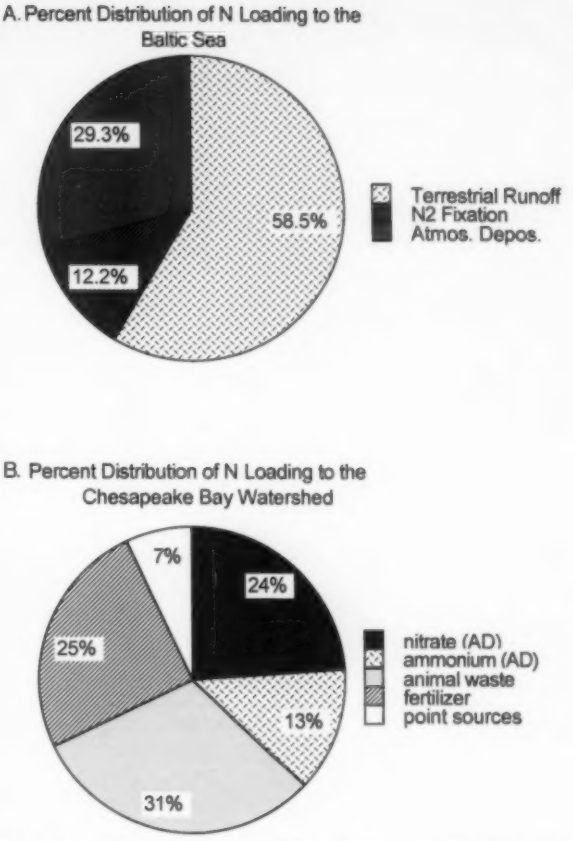

FIG. 2. Distributional graph depicting the percent distribution of dominant "new" N loading sources to the (A) Baltic Sea proper (data from Elmgren (1989) and V.N. de Jonge (personal communication)) and (B) the Chesapeake Bay watershed (AD indicates atmospherically derived) (data from Fisher and Oppenheimer (1991)).

Zika 1987; H.W. Paerl et al., in preparation), constituting from 10 to more than 30% of wet plus dry deposition collected at a North Carolina coastal location (H.W. Paerl et al., in preparation). Bioassay experiments conducted in receiving estuarine, Atlantic coastal and offshore waters indicate that a N fraction in addition to dissolved inorganic nitrogen (DIN) has been implicated in the enhancement of primary production (Paerl et al. 1990, Paerl 1993; Willey and Paerl 1993). It is likely that this "missing fraction" is ON. Rainfall-based ON collected in coastal Florida (Mopper and Zika 1987) and coastal North Carolina (M.L. Fogel and H.W. Paerl, unpublished data) revealed detectable concentrations of amino acids. Rainfall collected over New Zealand contains significant amounts of urea (Timperley et al. 1985). Although the sources of these ON constituents are not clear, recent studies indicate that both amino acids and urea can be readily assimilated at trace concentrations by marine phytoplankton (McCarthy 1972; Paul 1983; Flynn and Butler 1986; Antia et al. 1991; Paerl 1991).

Transit distances of ADN from sources to receiving waters can be substantial. It is well known, for example, that ADN encountered along the U.S. East Coast can originate at midwest powerplants and urban centers 1000 km or more away (Briblecombe and Stedman 1982; Jickells et al. 1982; Altshuler 1984;

TABLE 3. Annual estimates of ADN (wet + dry) fluxes in geographically diverse open-ocean, coastal, and estuarine regions. Data were obtained from GESAMP (1989). Estimates are conservative because they do not include dissolved or particulate organic N.

Location	Lat., long.	Measurement period	ADN flux (mmol $N \cdot m^{-2} \cdot yr^{-1}$)
		Western North Atlantic	
Bermuda	32°N, 65°W	Apr. 1980 – May 1984	10
Florida	24°N, 80°W	1984–85	15
North Carolina	37°N, 77°W	1984–85	25
Delaware	39°N, 75°W	1979–83	37
New York	41°N, 73°W	1979–83	34
Maine	44°N, 68°W	1984–85	27
Newfoundland	49°N, 55°W	1985–86	6.7
		Eastern North Atlantic	
U.K.	50°N, 5°W	1983–85	31
Norway	66°N, 14°E	1983–85	16.5
		North Sea	
U.K.	53°N, 0.5°E	1983–85	61
Netherlands	53°N, 7°E	1983–85	94
Germany	55°N, 8°E	1983–85	74
Denmark	56°N, 10°E	1983–85	65
Norway	59°N, 7°E	1983–85	78
		Baltic Sea	
Sweden	57°N, 18°E	1983–85	65
Finland	60°N, 21°E	1983–85	65
Russia	55°N, 21°E	1984–85	50
		Eastern Mediterranean	
France (Corsica)	42°N, 8°E	1984–86	52
		Indian Ocean	
Amsterdam Is.	38°S, 78°E	May 1980 – June 1986	6.9

Galloway et al. 1984; Duce 1986, 1991; Levy and Moxim 1987). Similarly, ADN in the Baltic and North seas largely originates from the British Isles and Western and Southern Europe (including France, Germany, and the Benelux) (Rodhe et al. 1980; Derwent and Nodop 1986; Soderlund 1987; GESAMP 1989; Prado-Fiedler 1990). This characteristic makes ADN and associated water quality issues regional, international, and perhaps global in nature, presenting a formidable problem from biogeochemical, ecological, and economic as well as environmental management perspectives. For example, on relatively short time scales (10–50 yr), ADN may be a more important problem than global warming in mediating "new" marine primary production and associated CO_2 flux.

Economic "hard choices" lie ahead in the control and management of ADN, For example, addressing this issue among the closely intertwined (and interdependent) economies of Europe is no enviable task. Some of the largest sources of atmospheric N pollution result from mainstay economic activities. Examples include dairy and farming concerns of the Netherlands, Belgium, and France, which generate large amounts of NH_3 and NH_4^+, industrial fossil fuel emissions from the U.K., Germany, and Eastern Europe, which are formidable NO_x emmiters, and large commercial urban centers (London, Paris, Frankfurt, Rome, Athens, etc.) in which automobiles are major NO_x contributors. In North America, similar long-distance, interstate, and international management issues exist. Consider, for example, the issue of Midwest urban coal-fired powerplants and automobile emissions as they affect Atlantic Seaboard N loading and eutrophication (Fisher and Oppenheimer 1991; Paerl et al. 1993).

While long-distance transport is a biogeochemical and management issue unique to ADN, it is not the only one. Atmospheric N deposition also manifests itself uniquely in terms of its delivery to and assimilation by receiving waters. These waters can be impacted either directly (by direct deposition onto N-sensitive lakes, rivers, estuaries, and coastal waters) or indirectly (by being deposited on watersheds, followed by subsequent discharge into receiving waters). Watersheds, tributaries, wetlands, and estuaries act as biological "filters," stripping and converting ADN as it transits to the coastal zone (Correll and Ford 1982; Kennedy 1984; Warwick and Hill 1988; Billen et al. 1991). The functional and quantitative roles that watersheds play in the processing of ADN are uncertain. As much

as 30–60% of ADN may be "lost" via denitrification in certain watersheds, prior to its discharge into coastal waters (Seitzinger 1988). Another ill-defined fraction of ADN may be incorporated by plants in the watershed; if incorporated by slow-growing trees, this constitutes long-term N and C storage (Warwick and Hill 1988). Berner and Berner (1987) estimated that of the total N delivered to land globally (including biological N_2 fixation), only 18% makes its way to coastal waters. In Chesapeake Bay, Fisher and Oppenheimer (1991) estimated that approximately 25–50% of watershed-based N loading (Fig. 2B) is delivered to the bay proper. The relative proportion of ADN retained and or "lost" by the watershed is dependent on geomorphology, soil characteristics, degrees of agricultural and forest coverage, urbanization, and industrial development within the watershed.

Another important factor, related to ADN impacts on coastal eutrophication, is the proportionality of watershed to water body areas and volumes. In contrast with terrigenous sources of N loading, such as wastewater and groundwater discharge and surface runoff which to varying extents are processed by watershed, wetlands, and estuarine "filters," ADN can directly impact receiving waters by impinging on surface waters "downstream" of N stripping zones. In the case of N-limited estuaries and wetlands, which can often quantitatively strip much of the biologically reactive N contained in transiting waters (Kennedy 1984; Nixon 1986), direct deposition can constitute a highly significant source of downstream and coastal N. In North Carolina coastal waters, ADN accounts for approximately 30–40% of "new" N loading in estuarine components, while it may contribute in excess of 50% of "new" N loading in full-salinity nearshore waters less impacted by terrigenous N inputs (Paerl et al. 1993). In certain water bodies, such as the Baltic and North seas, Western Mediterranean, and other coastal waters not heavily impacted by large rivers, estuaries, or other sources of terrigenous N loading, direct ADN deposition constitutes the main "new" N (i.e. nonrecycled, originating from outside the water body) source. It is estimated that for the Baltic and Western Mediterranean seas, as much as 40–50% of the "new" N loading may originate this way (Martin et al. 1989; Loye-Pilot et al. 1990; Prado-Fiedler 1990).

As "new" N sources, direct and indirect ADN inputs into coastal waters yield major biogeochemical and trophic implications. It is now well recognized that "new" primary and secondary production (that proportion of production supported by nonrecycled nutrient inputs) is heavily dependent on allochthonous N inputs and upwelling in the marine environment (Eppley and Peterson 1979; Legendre and Gosselin 1989). Aside from biological N_2 fixation, which is thought to support no more than 5–10% of oceanic (including estuarine) primary production (Capone and Carpenter 1982), and upwelling (i.e. reintroduction of remineralized nutrients from below the euphotic zone), approximately half the oceanic "new" production can be attributed to allochthonous N inputs (Eppley and Peterson 1979; Legendre and Gosselin 1989; Paerl 1993; Fig. 1).

Geographically, more than half of the oceanic "new" production resides in coastal and marginal shelf environments which account for only about 10% of the surface area (Mantoura et al. 1991; Wollast 1991). These environments are also considered major repositories for marine sediment accumulation and elemental (specifically C) burial (Walsh et al. 1985; Milliman 1991; Wollast 1991). Lastly, they are the major sites of marine eutrophication and nuisance phytoplankton bloom proliferation (Paerl 1988; Smayda 1989; Smetacek et al. 1991).

Addressing Regional and Global Atmospherically Driven Eutrophication: Experimental Approaches

From nutrient loading and nutrient-production perspectives, a compelling case can be made for implicating enhanced ADN in coastal eutrophication and nuisance bloom dynamics. At this time, however, what *direct* evidence exists to support this contention? In short, the answer is, not much! Bioassay-based evidence indicates that at natural dilutions, ADN stimulates primary production in N-depleted estuarine and coastal Atlantic Ocean waters (Paerl 1985; Paerl et al. 1990, 1993; Willey and Paerl 1993) (Fig. 3). This work is currently being extended to assess phytoplankton community compositional as well as biomass before and after (in response to) acute and chronic (seasonal and interannual) depositional events (high-N-loaded continental versus low-N-containing oceanic storm events).

A particularly promising complementary technological approach to bioassays and field monitoring efforts is the use of stable N isotope analyses of sources and fates of ADN sources. The dominant sources of ADN have stable N isotope ratios ($\delta^{15}N$) or "signatures" distinct from terrigenous N input sources and regenerated N (Table 4). The "uniqueness" of ADN $\delta^{15}N$ "signatures" provides hope that this feature may be used to identify and assess distinct sources of N in terms of their roles in supporting coastal "new" production, eutrophication, and phytoplankton blooms. Applications of this approach to the topics discussed here are provided by Fogel and Paerl (1991) and Paerl et al. (1993).

In experimentally addressing the impacts of ADN on marine production and trophic alterations, it must be recognized that ADN manifests itself in distinct ways, each of which perhaps leads to unique sets of biological responses. In terms of direct deposition effects, wet deposition events, such as local storms and larger scale frontal passages, should be considered as acute impacts, that is, they can lead to substantial, albeit brief and intense, N enrichment to surface waters. In contrast, dry deposition is a chronic phenomenon, characterized by relatively low but persistent levels of N input over time. A prioiri, one would expect these two ADN forms to yield discrete and distinct impacts on phytoplankton primary production and community composition over time and space. To address and evaluate chronic versus acute N loading events, both response assays and monitoring efforts should be appropriately designed. It is likely that long-term bioassays needed to address chronic N loading may suffer from previously discussed complications, including forced isolation of assay from ambient water, undesirable surface or "bottle effects," lengthy modification of turbulence, and food chain (grazer and higher trophic levels) alterations. Alternative experimental strategies are needed. In the case of these ADN deposition events, increased emphasis should be placed on field nutrient–production monitoring and interpretational studies.

Productivity responses to ADN/frontal events may range from a day to several weeks, depending on the timing of and relative enrichments from direct and indirect (runoff) inputs. This necessitates short- as well as long-term detection and characterization of productivity responses, integrated over large areas, a task ideally suited for multispectral (to detect diagnostic phytoplankton pigments such as chlorophylls, carotenoids, and phycobilins; see below) aircraft- and satellite-based remote sensing (SeaWiFS). Of specific relevance is the proposed SeaWiFS satellite remote sensing platform, to be launched in the spring of 1994. It will be capable of discrete spectral analyses

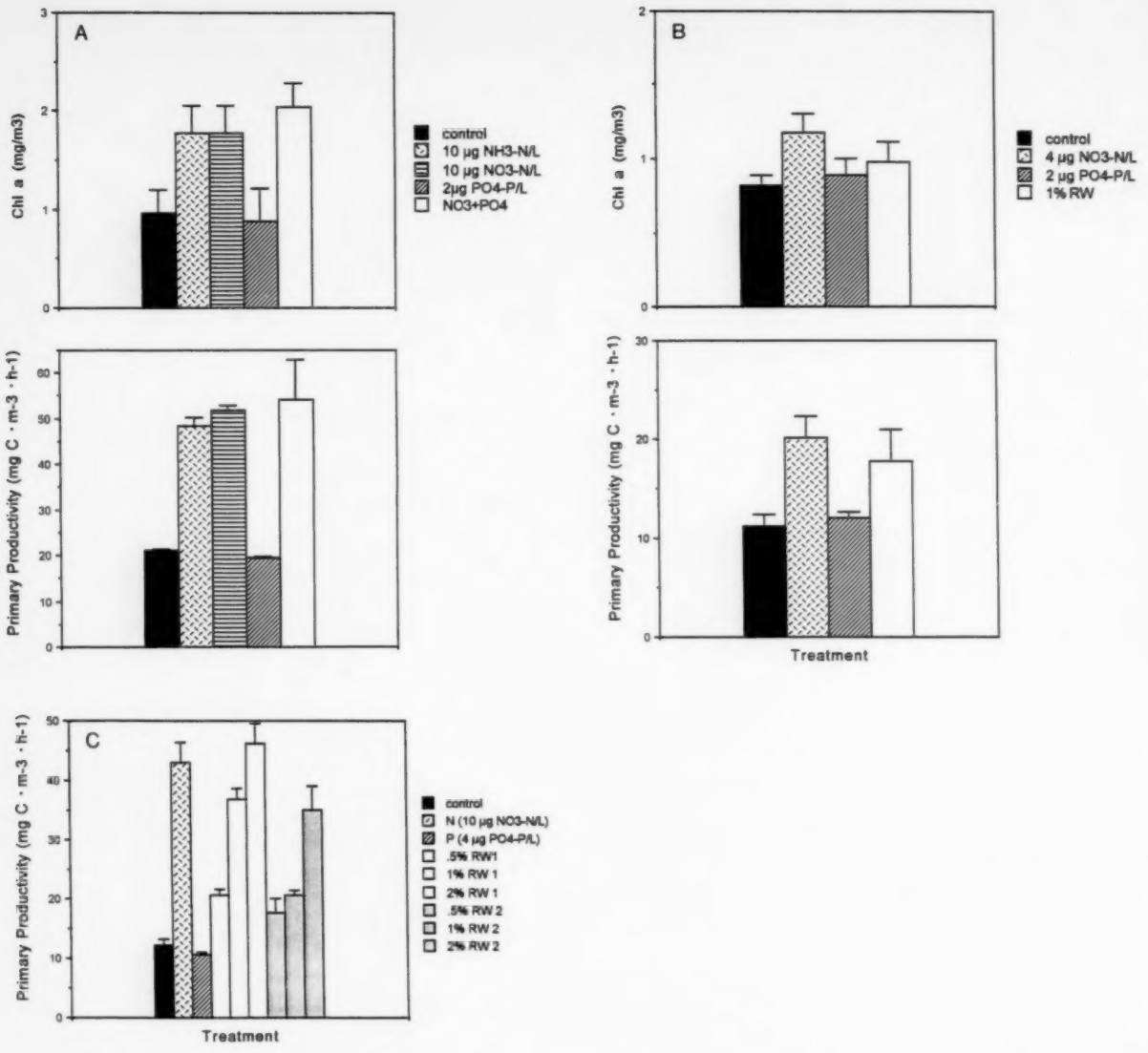

FIG. 3. Results from (A and B) 3-d mesocosm (~600 L "Hydrocorral") and (C) 4-d microcosm (~4 L "Cubitainer") bioassays illustrating the stimulatory impacts of specific N sources (NO_3^-), including N-enriched rainfall, on primary productivity ($^{14}CO_2$ fixation) and phytoplankton biomass (as chlorophyll *a*) in N-deplete North Carolina coastal Atlantic Ocean water (Bogue Sound, N.C.). Individual nutrients were added, in triplicate, at final concentrations shown in the legend. Rainfall was added at naturally occurring dilutions, calculated from salinity changes immediately following significant (>1 cm) rainfall events. Bioassays were incubated under natural irradiance and temperature conditions. For additional experimental details, please refer to Paerl (1985), Paerl et al. 1990, and Willey and Paerl (1993). Error bars represent standard deviation. (A) Low concentrations of DIN representative of N enrichment experienced during rainfall in surface waters led to significant (one-way ANOVA, $p < 0.01$) stimulation of both primary production and chlorophyll *a* production. Representative PO_4^{3-} additions yielded no stimulation. Growth responses to N plus P additions were not significantly different from N additions alone. (B) Final DIN concentration in a 1% rainwater addition was 3.4 µg N·L^{-1}. This N enrichment was very low, yet substantial enough to significantly (one-way ANOVA, $p < 0.01$) enhance primary production. (C) N additions consistently and significantly (one-way ANOVA, $p < 0.01$) stimulated production, as did the various rainwater additions, while P additions failed to do so. Rainfall from two storm events was added. The first (RW 1) was an "acid rain" event (pH 3.09), while the second (RW 2) was a more neutral rain event (pH 5.21). The former yielded a DIN concentration of 8.1 µg N·L^{-1} (at 1% dilution), the latter 5.2 µg N·L^{-1}.

with high degrees of sensitivity and resolution. Field sampling coupled to rapid pigment/productivity assays (Paerl et al. 1990, 1993) indicates that productivity responses to frontal passages are readily detectable (i.e., a doubling to tripling of chlorophyll *a* concentrations and rates of primary productivity). The spatial and temporal coupling of such meteorological events to pulses and patches of primary production is of critical importance in determining annual primary production and CO_2 flux in these waters. A need exists to functionally separate the contemporaneous impacts of autochthonous-driven (upwelling and

regenerated N supplied to surface waters via mixing) and allochthonous-driven (ADN and runoff) primary production resulting from frontal passages. We must also evaluate this nutrient-production interaction on regional and global synoptic scales (in heavily impacted waters such as the Baltic Sea, North Sea, Sea of Japan, Mediterranean Sea, etc.). To this end, utilization of and field coordination with SeaWiFS (and other multiband spectral remote sensing systems) imagery are essential and well suited for examining both acute (days) and chronic (weeks to months) impacts of ADN in coastal and offshore waters.

As a field verification technique for remotely sensed phytoplankton pigments, high-performance liquid chromatography (HPLC) has proven effective in rapidly separating photosynthetic pigments, prior to spectrophotometric and fluorometric detection (Mantoura and Llewellyn 1983; Bowles et al. 1985). When coupled to real-time, multiwavelength, rapid-scanning capabilities, such as those provided by a photodiode array spectrophotometer (PDAS), HPLC offers the ability to rapidly and accurately distinguish and quantify pigments within monotypic and mixed algal assemblages (Paerl and Millie 1991). In this manner, certain bloom taxa known to contain unique pigments and combinations of pigments (such as diagnostic carotenoids, phycobilins, specific combinations of chlorophylls *a*, *b*, and *c*, and chlorophyll derivatives) may be identified and potentially quantified. Additionally, HPLC-PDAS, when coupled with autoinjectors and driven by state-of-the-art computer software, is amenable to large-scale characterization and monitoring programs (NSF's JGOFS; EPA's Toxics and Lakes Survey). Because phylogenetic distinctions among algae are often based on the presence or absence of chlorophylls, carotenoids, and phycobilins, this tool provides a means for rapid (~20 min per run) characterization of phylogenetic groups and monitoring changes in community composition, thereby providing accurate and cross-calibratable (with remote sensing) information concerning correlation of species assemblages with physical/chemical limitations and production. HPLC-PDAS is currently being used in assessments of trophic states of estuarine, coastal, and open-ocean ecosystems in relation to environmental perturbations (including nutrient loading attributable to ADN).

The recent global "epidemic" of toxic and noxious phytoplankton blooms in nutrient-enriched waters has underscored the need for large-scale satellite/airborne remote imagery for monitoring and potentially predicting bloom intensity and distribution. Toxic and noxious blooms are often monospecific and obtain relatively high biomass values. The continued development of high-resolution spectral resolving instruments (i.e., SeaWiFS) will improve multispectral pigment scanning capabilities, thereby facilitating the acquisition of spectral reflectance data based on pigments in addition to chlorophyll *a* (Millie et al. 1992). HPLC-PDAS will serve a quantitative and qualitative confirmative role in ground truthing for the remote sensing of algal reflectance signatures in response to nutrient loading events such as those discussed here. The coupling of high-resolution remote imagery with accurate pigment signatures of diverse phytoplankton will provide a heretofore unrecognized calibration of reflectance signatures (Millie and Kirkpatrick 1992) and will test the utility of remote sensing technology to accurately assess local, regional, and global estimates of spatial/temporal dynamics of phytoplankton production, community structure in response to physical/chemical forcing events such as wet/dry atmospheric nutrient inputs, terrestrial inputs, altered circulation patterns, upwelling, etc. Additionally, the use of remotely sensed environmental and biogeochemical information is the key to characterizing hydrographic features that control bloom dynamics (Tester et al. 1989).

TABLE 4. Typical $\delta^{15}N$ values for particulate and dissolved N in U.S. estuarine/coastal waters (Velinsky et al. 1989; Fogel and Cifuentes 1993; Paerl et al. 1993).

	$\delta^{15}N$
Particulate N	
Estuarine phytoplankton	+6.0 to +20.0
Coastal phytoplankton	+6.0 to +10.0
Marine cyanobacteria	−2.0 to +4.0
Terrestrially derived N	−2.0 to +5.0
Dissolved inorganic N	
N_2 in air	0.0
Remineralized NH_4 (estuarine)	+10.0 to +16.0
Sewage effluent	
NH_4	+5.0 to +11.0
NO_3	+5.0 to +10.0
Agricultural land runoff	+8.0 to +10.0
Fertilizers	−2.0 to +2.0
Atmospheric deposition	
NH_4	−13.0 to +2.0
NO_3	−5.0 to +2.0

Concluding Remarks

Regionally and globally, evidence is mounting that enhanced N loading is closely linked, in time and space, to eutrophication and an associated "epidemic" of nuisance phytoplankton blooms in N-limited estuarine and coastal waters. While efforts are being undertaken to control identifiable terrigenous point and nonpoint N loading sources currently impacting these waters, atmospheric N deposition remains enigmatic and uncontrolled. This hitherto uncharacterized N source appears to be increasing in magnitude and proportion to other sources and geographic distribution. It is suspected that previously pristine coastal and offshore waters are now impacted by this source to the extent that in some regions (Mediterranean and Baltic), ADN appears to be the single largest source of "new" N supporting eutrophication and meeting nutrient thresholds for blooms.

Identification of ADN sources and their fates is complicated by the fact that atmospheric emissions can travel large distances and hence impact waters not necessarily adjacent to sources. Stable N isotope analyses may prove instrumental in identifying ADN sources and their biogeochemical (flux and cycling) as well as trophic (food web) fate in the marine environment.

Functional links between ADN and eutrophication are compounded by complex interactions of N and other limiting nutrient (P, Fe, and Si) and physical-chemical interactions (mixing and upwelling), over time and space, between nutrient sources and receiving waters. To this end, remote sensing, coupled to recently developed diagnostic production and nuisance phytoplankton bloom detection techniques, will prove invaluable and attainable, once a new generation of high-sensitivity, high-spectral- and high-spatial-resolution remote sensing instrumentation for aircraft and satellites becomes operational.

During the course of researching and writing this synthesis, it has become evident that our conceptual, experimental, and management views of and approaches to coastal nutrient–production interactions are in need of reevaluation and revision. Based on the expanding domains of nuisance blooms and associated nutrient cycling and trophic changes, emphasis should be placed on regional, if not global, air-sea and land-sea interactions. In the case of ADN, these interactions transcend international and continental boundaries. A formidable but essential challenge lies ahead as we enter the next century in bridging basic mechanistic with applied research as we attempt to clarify and manage this and other emerging nutrient–production issues that affect coastal biogeochemical and resource dynamics.

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Climate and Exceptional Fish Production off the West Coast of North America

Richard J. Beamish

Department of Fisheries and Oceans, Biological Sciences Branch, Pacific Biological Station, Nanaimo, BC V9R 5K6, Canada

Beamish, R.J. 1993. Climate and exceptional fish production off the west coast of North America. Can. J. Fish. Aquat. Sci. 50: 2270–2291.

From 1976 to 1978 there was a change in the climate over the North Pacific Ocean. The Aleutian Low intensified and there was a warming of the sea surface adjacent to North America and a cooling offshore. Associated with this change was a period of exceptional fish production. Strong year classes and above-average survival occurred for many commercially important species all along the west coast of Canada and the United States. Trends in total salmon catches increased primarily from increased salmon production in Alaska. Some stocks of maturing pink (*Oncorhynchus gorbuscha*), coho (*O. kisutch*), and chinook salmon (*O. tshawytscha*) also had above-average growth in 1977. A majority of commercially important nonsalmon species that spawned from California to the Bering Sea and have a wide range of life history types also had exceptionally strong year classes from 1976 to 1978. The exceptional survival appears to be related to improved ocean productivity caused by changes in the intensity of the Aleutian Low.

De 1976 à 1978 a eu lieu une modification du climat au-dessus du Pacifique nord. La dépression des Aléoutiennes s'est intensifiée, et on a observé un réchauffement de la surface de la mer à proximité de l'Amérique du Nord, et un refroidissement au large. Ce changement s'est accompagné d'une période de production de poissons exceptionnelle. De fortes classes annuelles et un taux de survie au-dessus de la moyenne ont été notés pour de nombreuses espèces commercialement importantes tout le long de la côte ouest du Canada et des États-Unis. Les tendances des prises totales de saumon ont augmenté, ce qui est dû principalement à l'accroissement de la production salmonicole en Alaska. Certains stocks de saumon rose (*Oncorhynchus gorbuscha*), coho (*O. kisutch*) et quinnat (*O. tshawytscha*) ont aussi présenté en 1977 une croissance au-dessus de la moyenne. La majorité des espèces commercialement importantes de poissons autres que des salmonidés, qui fraient de la Californie à la mer de Béring et ont des cycles biologiques très divers, ont aussi connu des classes annuelles exceptionnellement fortes de 1976 à 1978. Ce taux de survie exceptionnel semble être lié à une amélioration de la productivité marine causée par la modification de l'intensité de la dépression des Aléoutiennes.

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Understanding recruitment is fundamental to understanding how to sustain fisheries. Environmental influences on recruitment receive less attention in traditional population dynamics because the biological processes regulating recruitment are generally poorly understood. Neglecting or averaging environmental effects on stock size oversimplifies the population dynamics process and will ultimately lead to management problems. Identifying close relationships between recruitment strength (year-class strength) and environmental conditions probably will help clarify the population dynamics process and may even improve the accuracy of models that forecast long-term abundance trends.

In this report, I identify a period from 1976 to 1978 when major changes in fish abundance occurred and then relate the abundance changes to changing environmental conditions. I examined catch trends of many of the important west coast marine fishes in the commercial catches of Canada and the United States. Some data series appear short or incomplete because they do not begin until the early 1970s or because they end in the early 1980s, as long-lived species such as Pacific halibut (*Hippoglossus stenolepis*) or Pacific ocean perch (*Sebastes alutus*) are not recruited to the commercial fishery until they are 8-12 yr old. Thus, information on the year-class strength of some commercially important species is available only from the early 1970s to the early 1980s.

Brief descriptions of the climate and the oceanography of the North Pacific Ocean and the Strait of Georgia are included to provide background information for the discussion of environmental changes that occurred in 1976 and 1977. Descriptions and discussions of climate and oceanography are for the winter period because most demersal fishes off the west coast of North America spawn in the winter (Hart 1973) and it is known that there is a relationship between salmon production and the winter climate over the northern North Pacific Ocean (Beamish and Bouillon 1993).

Climate and Oceanography in the North Pacific

During the winter, the climate over the North Pacific Ocean is dominated by the Aleutian Low (Fig. 1), a deep extensive low pressure area that develops late in the year (Trenberth 1990; Beamish and Bouillon 1993). The low begins to break down in the spring and is replaced in the summer by an area of extensive high pressure. The major ocean currents are generated by the

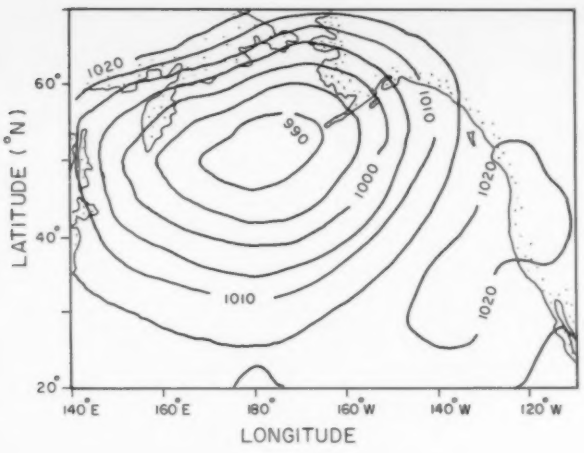

FIG. 1. Aleutian Low that formed from December 1976 to February 1977. Area less than 1000 kPa indicates the extent of this extreme low (from Hamilton 1984). The contour interval is 5 kPa.

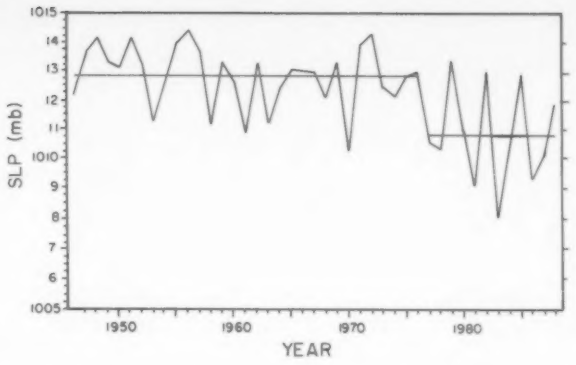

FIG. 2. Mean sea level pressure in the North Pacific Ocean from November to March showing the change in the pattern of the winter climate that occurred in 1976 (from Trenberth 1990).

resulting surface winds, with the strength of the currents related to the strength of the winds. Seasonal differences in the direction of the winds determine if surface currents (called Ekman transport) flow inshore or offshore (Dodimead 1984; Freeland et al. 1984).

In the North Pacific, the ocean circulation consists of a clockwise-flowing Central Pacific gyre and a counter-clockwise-flowing Alaskan gyre. The boundary between these two currents, the Subarctic Current, divides into two branches as it nears the coast of North America at about latitude 45–50°N. One branch, the California Current, flows south and the other, the Alaska Current, flows north off the coasts of British Columbia and Alaska. As the Aleutian Low develops in the fall, the circulation around the Alaska gyre intensifies (Thomson 1981). During periods of strong Aleutian Lows, there is strong onshore transport of surface currents (the Ekman layer) and intense downwelling along the coast as far south as central California. This mass of water flowing towards the coast must be replaced by water from below. Hence, there is a shallowing of the upper mixed layer to the north in the central Pacific Ocean (Thomson 1981) bringing cooler, nutrient-rich water to the surface.

Climate and Oceanographic Changes in the Late 1970s

The Aleutian Low of 1976–77 (Fig. 1) was the most intense low since 1940–41 (Beamish and Bouillon 1993). From December 1976 through May 1977, most of the northern North Pacific Ocean was dominated by this area of intense low pressure. This intense low also signaled a change in the pattern of winter (November–March) low pressures (Trenberth 1990). Trenberth (1990) showed that from 1956 until 1976, the mean sea level pressure over the North Pacific Ocean was approximately 1012.8 mbars (1 mbar = 100 Pa). After 1976, (1977–88) it was approximately 1010.8 mbars, a drop of 2 mbars over the vast area of the North Pacific Ocean (Fig. 2). The centre of the Aleutian Low moved farther east and became deeper by an average of 4.3 mbars for the five winter months and deeper by 7–9 mbars in January.

Another indication of a climate change over the North Pacific in the late 1970s is the change that occurred in sea surface temperatures. Sea surface temperatures in the Northeast Pacific Ocean have two general patterns: one, where sea surface temperatures along the coast of North America are below normal when temperatures farther offshore are above normal and a second type that is the opposite (McLain 1984). These patterns are most obvious in the winter. The pattern oscillated over the years 1957–76 (Fig. 3). In the summer of 1976, however, it changed from having below-normal sea surface temperatures adjacent to shore to having above-normal sea surface temperatures close to shore (McLain 1984). The increase in sea surface temperatures lasted until at least the mid-1980s and was most dramatic in the Northeast Pacific Ocean (Chelton 1984; Fig. 4). A time series of sea surface temperatures using a running average of three months shows that a period of lower than usual sea surface temperatures came to an end in 1976 (Fig. 4).

The abrupt change in climate in 1976 was clearly shown by Ebbesmeyer et al. (1991) using combined records of 40 environmental variables that reflected environmental conditions for 1968–84 off the west coast of North America in the vicinity of Puget Sound. The changes were identified in the Gulf of Alaska (Royer 1989) and off California (Bakun 1990). The climate shift started a new climate regime over a vast area of the Pacific Ocean and North America (Kerr 1992). In general, the period 1977–86 featured very large air temperature anomalies in the North Pacific basin. There was a general warming exceeding 1.5°C over Alaska (Fig. 5) and a cooling of less than 0.75°C in the central and western North Pacific (Trenberth 1990). This pattern of warming over Alaska and cooling in the central and western North Pacific would be expected with more intense Aleutian Lows because warmer, moister air would be carried along the west coast of North America into Alaska. At the same time, cooler, southward flowing air would be found in the central and western North Pacific.

In British Columbia, the winter of 1975–76 was one of the warmest and wettest on record. Records from a number of locations throughout British Columbia indicate total precipitation in the winter of 1975-76 as the highest on record over the period 1939-89 (Fig. 6A). There was also a change in the long-term precipitation pattern. Beginning in the mid-1970s, average annual precipitation, measured from June in one year to May in the next, increased near the coast (Fig. 6B), and since 1980,

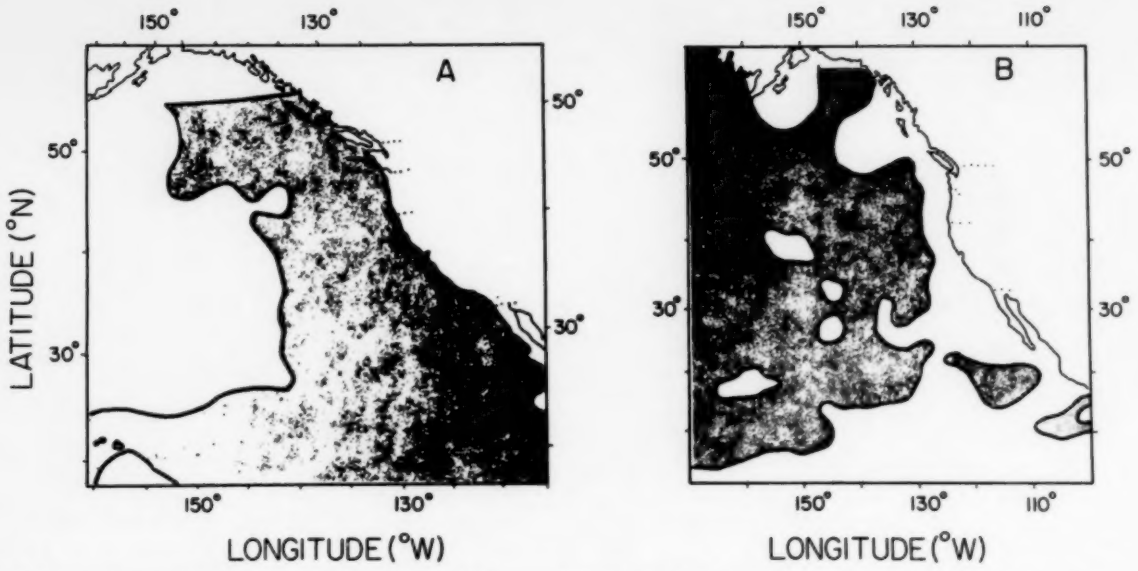

FIG. 3. Pattern of sea surface temperature anomaly (°C) for (A) January 1972 showing below-normal temperatures (shaded area) close to shore and (B) January 1983 showing above-average temperatures close to shore (unshaded area) (from McLain 1984).

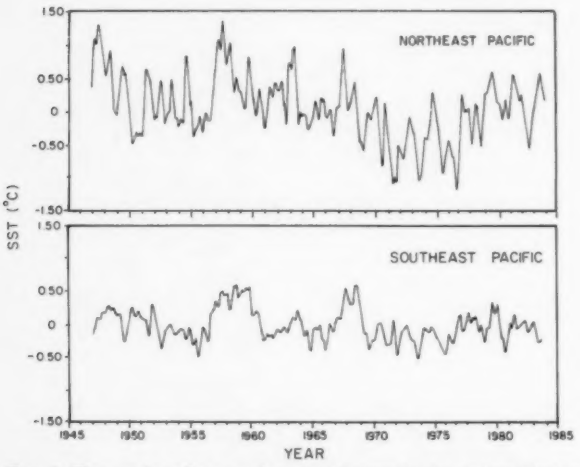

FIG. 4. Time series of sea surface temperature in the eastern Pacific Ocean presented as a 3-mo running average showing the trend to increasing temperatures between 1972 and 1979 in the Northeast and Southeast quadrants of the North Pacific Ocean (from Chelton 1984).

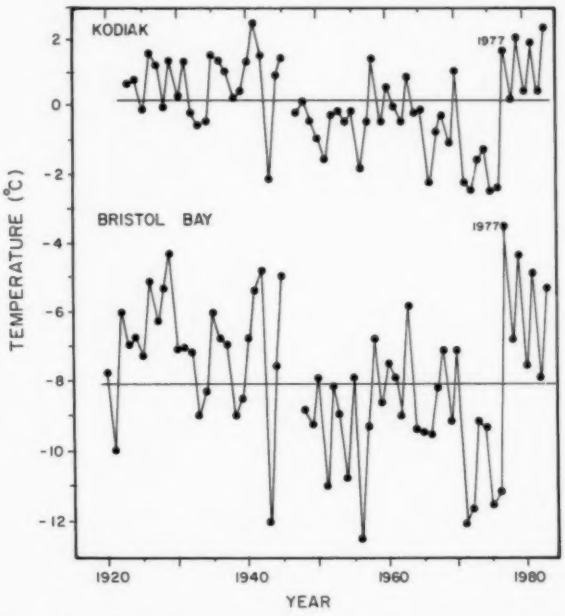

FIG. 5. Average winter (November–March) air temperature for Kodiak and Bristol Bay, Alaska, showing abrupt increases in 1977 (from Rogers 1984).

precipitation has been above the long-term average each year. In contrast, discharge from the Fraser River, the largest and most important river in British Columbia, was the highest on record in 1976 (Fig. 7) but declined after that year. This decline in discharge appears to be related to a decline in snowpack in the interior of British Columbia that appears to have started in 1977. The annual average snowpack from 24 stations within the Fraser River Basin from 1977 to 1988 was 22% less than the annual average snowpack from 1960 to 1976 (D. Moore, Department of Geography, Simon Fraser University, Burnaby, B.C.). It appears, therefore, that precipitation along the coast increased, but decreased inland. Because most water in the Fraser River comes from the interior of British Columbia, the annual discharge declined.

The Strait of Georgia is the most important marine area off the west coast of Canada and one of the most important salmon-producing areas in the North Pacific Ocean. The physical oceanography is mainly affected by runoff and nutrient-rich inflowing deep water of oceanic origin (Tully and Dodimead 1957). The major source of runoff is the Fraser River, contributing almost 80% of the runoff (Waldichuk 1957).

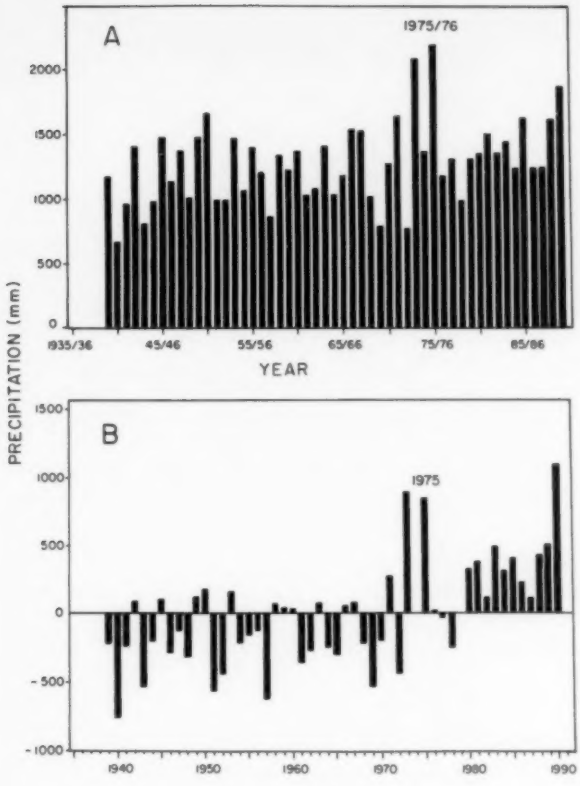

FIG. 6. (A) Total precipitation (rain and snow) for October–March and (B) precipitation anomaly for June–May at Hope, B.C. (data from the Temperature and Precipitation Abstracts, Atmospheric Environment Service, Canada).

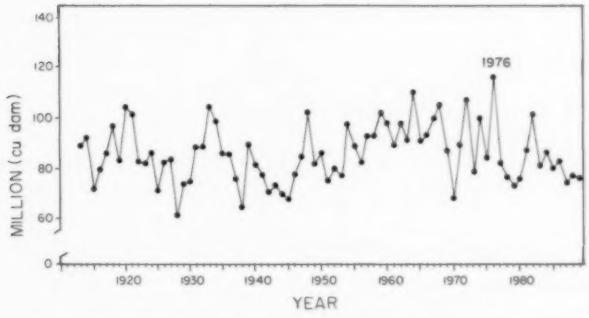

FIG. 7. Total annual discharge from the Fraser River (Environment Canada 1990).

Bottom water temperatures have been recorded in the Strait of Georgia by the Department of National Defence since 1969. The annual cycle of temperature change reflects the change in the volume of cool oceanic water that enters the Strait of Georgia to replace surface water transported out as a consequence of Fraser River discharge. The annual temperature cycle of the bottom water was relatively uniform until 1976 (Fig. 8), but from 1976 until 1980 the cycle was disrupted. After 1980, a new cycle was established; average annual bottom water temperatures were

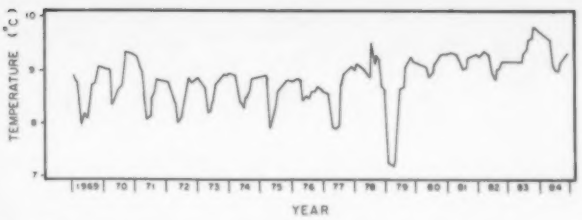

FIG. 8. Bottom water temperatures in the Strait of Georgia (data courtesy of R.E. Thomson, Institute of Ocean Sciences, Sidney, B.C.). Data from 1985 to the present were not available.

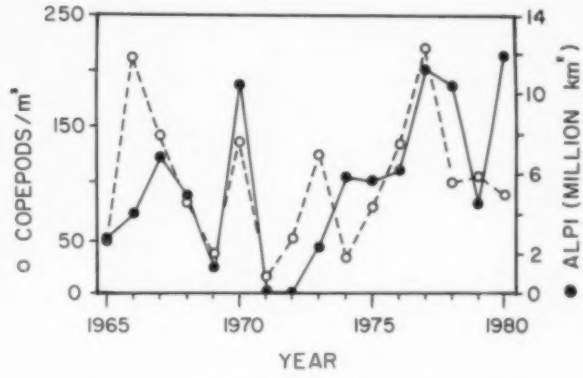

FIG. 9. Relationship ($r = 0.50$, $p < 0.05$) between copepod abundance at Ocean Station "P" (broken line, open circles) (from Fulton 1983) and the Aleutian Low Pressure Index (ALPI) (solid line, solid circles) (from Beamish and Bouillon 1993).

warmer and there was less annual fluctuation. The timing of the change in this annual pattern of bottom water temperature coincides with the other changes in climate and oceanography, including the record discharge from the Fraser River.

The longest continuous time series of plankton abundance changes in the central North Pacific Ocean, collected at Ocean Station "P" (Fulton 1983), was discontinued in 1980. The maximum average abundance of copepods, for the period most larval fish would be feeding on copepods (March–May), occurred in 1977 (Fig. 9). There was a significant relationship between the intensity of the Aleutian Low and copepod production at Ocean Station "P" (McFarlane and Beamish 1992; Beamish and Bouillon 1993; Fig. 9). The average annual production of copepods increased 1.5 times from 1976 to 1980 compared with the average from 1965 to 1975. Estimates of zooplankton biomass in the central subarctic Pacific Ocean doubled from the 1956 to 1962 period to the 1980 to 1989 period (Brodeur and Ware 1992), indicating that the increase in copepod abundance that occurred in the late 1970s at Ocean Station "P" probably persisted into the 1980s. Because copepods are the dominant species in the zooplankton (McFarlane and Beamish 1992) and a principal food for many marine species, there is a clear link between the intensification of the Aleutian Low and fish production.

It is clear that the climate change that began in 1976 was a major event all along the west coast of North America that was associated with increases in primary and secondary production on a large scale. Associated with these changes were major changes in fish abundance.

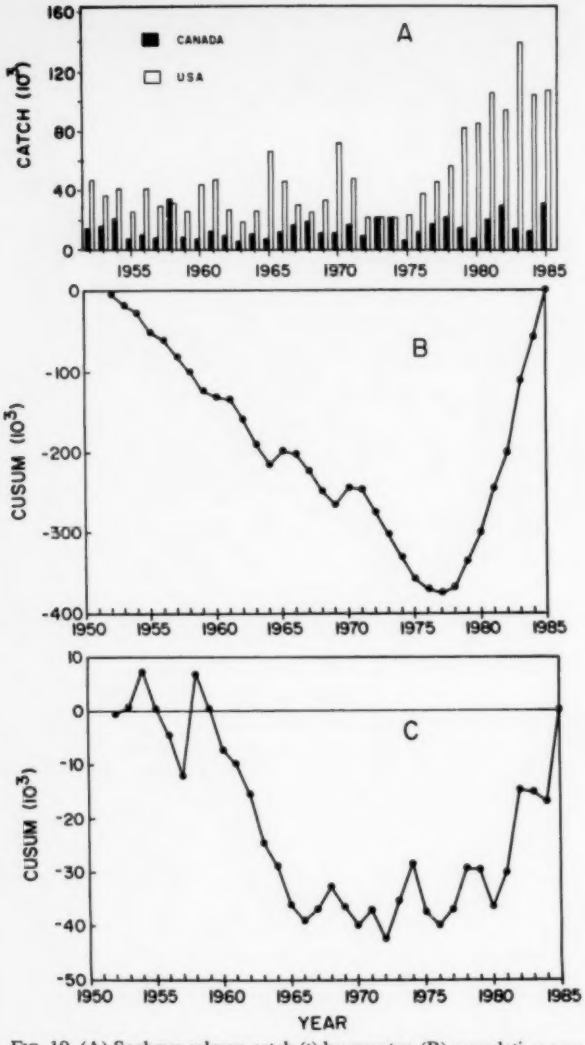

FIG. 10. (A) Sockeye salmon catch (t) by country, (B) cumulative sum of sockeye salmon catch (t) by the United States, 1952–85, showing the change in trend beginning in 1977, and (C) cumulative sum of sockeye salmon catch (t) by Canada, 1952–85 (INPFC 1979, 1977–89).

Changes in Salmon Abundance

Pacific salmon constitute a group of important species in the commercial fishery off the west coast of Canada and the United States and as a group are documented by one of the longest and most reliable data bases. Trends in all-nation catches of pink (*Oncorhynchus gorbuscha*), chum (*O. keta*), and sockeye salmon (*O. nerka*) changed in the late 1970s from about 1977 to 1979 (Beamish and Bouillon 1993). Average catches in the time series from 1925 to 1988 went from historic lows in the early 1970s to levels in the late 1980s that were close to the historic highs. In this report, I looked for stock-specific evidence of the timing and magnitude of this change in important North American commercial salmon fisheries. I reviewed the evidence that indicated that trends in the catches of salmon changed in the late 1970s and I also included biological and related information

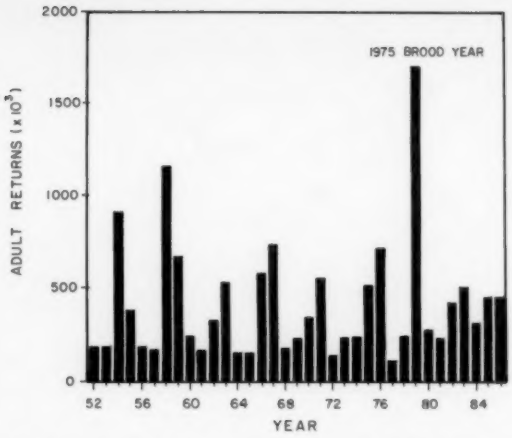

FIG. 11. Total return of adult sockeye salmon to the Stellako River (catch + spawning adults) (from Cass 1989). This stock is one of the Fraser River stocks that had exceptional adult returns in 1979.

to show that changes in size and behaviour were associated with the trend of increasing salmon production.

Sockeye Salmon (*Oncorhynchus nerka*)

Trends in catches can be demonstrated using cumulative sum charts (CuSum) charts (Murdoch 1979). CuSum plots use information contained in a time series to identify when changes in catch occurred. The slope of the graph is a measure of the mean trend, and a change in the slope indicates a change in the trend.

Ages are assigned to salmon to indicate the year of spawning. These ages are not equivalent to ages assigned to most other fishes and are not directly comparable with ages of nonsalmon species in this article. In most cases, sockeye salmon that entered the sea in 1977 were the 1975 brood year or fish that spawned in 1975. Once in salt water, most sockeye salmon spend 2 yr (winters) at sea before returning to spawn. Therefore, fish returning from the 1975 brood year in 1979 year are considered to be age 4.

Most of the increase in total North American salmon catch in the mid-1980s resulted from increased sockeye salmon catches in Alaska (Fig. 10A). The change in catch trends in Alaska occurred about 1977 (Fig. 10B). The trend in Canadian catches was more variable, changing approximately in 1959, 1966, and 1980 (Fig. 10C), with the increase in the mean catch beginning in the early 1980s.

In British Columbia, there are two major areas that produce sockeye salmon: the Skeena River drainage and the Fraser River drainage. A major difference between the Skeena River stocks and the Fraser River stocks is the presence of strong cycles of abundance in the Fraser River (Vernon 1982). The reason for the cycles is unknown, but their existence requires that survival comparisons be made for individual stocks or cycle years. Most sockeye salmon from the Fraser River that went to sea in 1977 (1975 brood year) were produced from some of the smaller stocks or from the less productive years of the larger stocks. Using the catch estimates compiled by Cass (1989), strong year classes (brood years) were produced for about one half of these stocks (Fig. 11). The average number of adults that return for each female spawner for all Fraser River stocks is 4.88 (Cass 1989). The return per spawner for the 1975 brood year or all the sockeye from the Fraser River that went to sea in 1977 was 6.73,

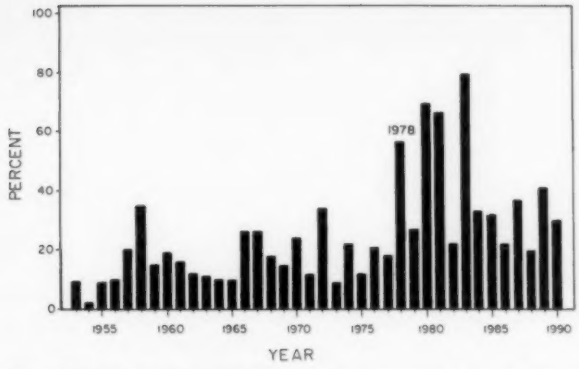

FIG. 12. Percent of Fraser River sockeye salmon returning to the Fraser River through Johnstone Strait (data courtesy of the Pacific Salmon Commission, Vancouver, B.C.). A high proportion returned through Johnstone Strait in 1978, 1980, 1981, and 1983.

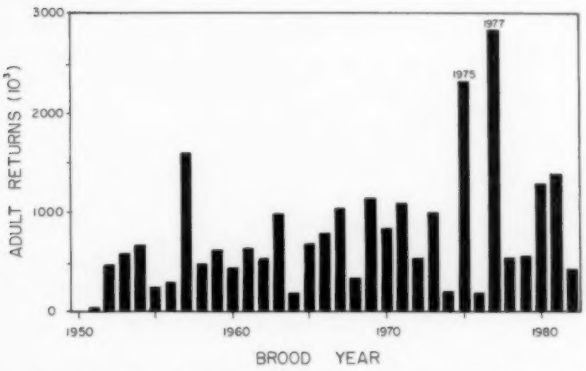

FIG. 13. Estimated number of age 4 sockeye salmon (catch + escapement) returning to the Skeena River (data courtesy of M. Henderson and R. Diewert, Department of Fisheries and Oceans, Vancouver, B.C.).

the highest survival rate for this cycle since the 1955 spawning. The egg to smolt survival for the Chilko River stock in the Fraser River was almost average for the 1975 brood year (Cass 1989). However, the smolt to adult survival was above the long-term average, indicating that for this stock the improved survival probably occurred in the sea.

The behaviour of sockeye salmon returning to the Fraser River changed in 1978. Sockeye salmon can return through Juan de Fuca Strait or through Johnstone Strait and through the Strait of Georgia. The percentage returning through Johnstone Strait has been estimated since 1953 (Fig. 12). In 1978, the percentage increased, and it increased further in 1980, 1981, and 1983.

In the Skeena River, sockeye salmon going to sea in 1977 and 1979 had above-average survival. The returns from fish that entered the ocean in 1977 (the 1975 brood year) were the highest since 1950 (Fig. 13). The returns from fish that went to sea in 1979 (1977 brood year) also were exceptionally high (Fig. 13).

Sockeye salmon in Alaska live in fresh water for 2 or 3 yr and return to fresh water after 2 or 3 yr in salt water. Adults, therefore, can be 4, 5, or 6 yr old, but most adults are 5 yr and most of these remained in fresh water for 2 yr. The returns from sockeye that

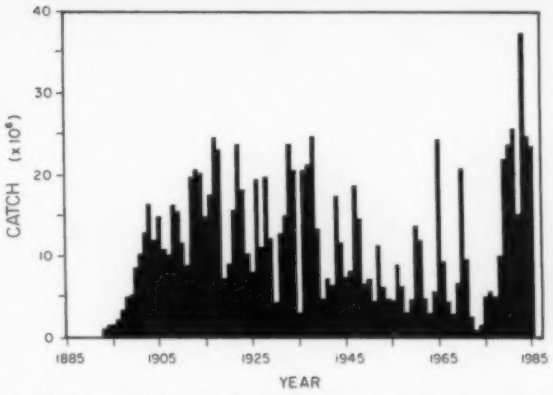

FIG. 14. Commercial catch of sockeye salmon in Bristol Bay, Alaska, showing an abrupt increase in catch beginning in the late 1970s (from Minard and Meacham 1987).

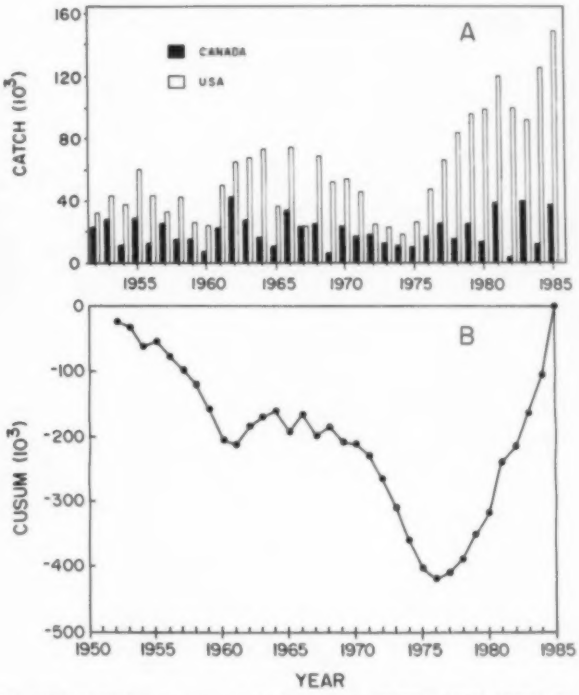

FIG. 15. (A) Pink salmon catch (t) by country (INPFC 1979, 1977–89) and (B) cumulative sum of the pink salmon catch (t) by Canada and the United States, 1952–85, showing the increasing abundance trend that started in 1977.

went to sea in 1977 produced record high catches (Minard and Meacham 1987; Fig. 14), particularly in the Kvichak River, the largest producer of sockeye salmon in the catch. There is a 5-yr cycle in the Kvichak River that produced strong year classes in 1975 and 1980. Thus, fish from the 1975 brood year that went to sea in 1977 and 1978 would be expected to produce another strong cycle year. However, fish from the much weaker 1974 brood year that remained in fresh water for 3 yr and went to sea in 1977 had the highest return per spawner on record (estimated

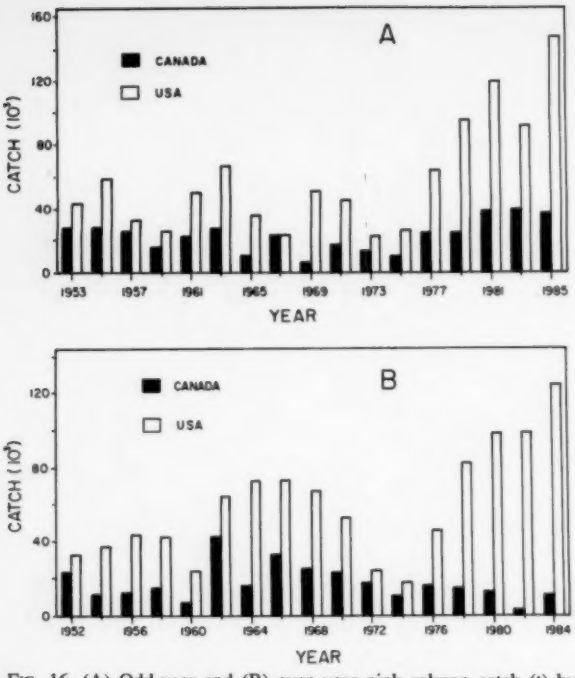

FIG. 16. (A) Odd-year and (B) even-year pink salmon catch (t) by country (INPFC 1979, 1977–89).

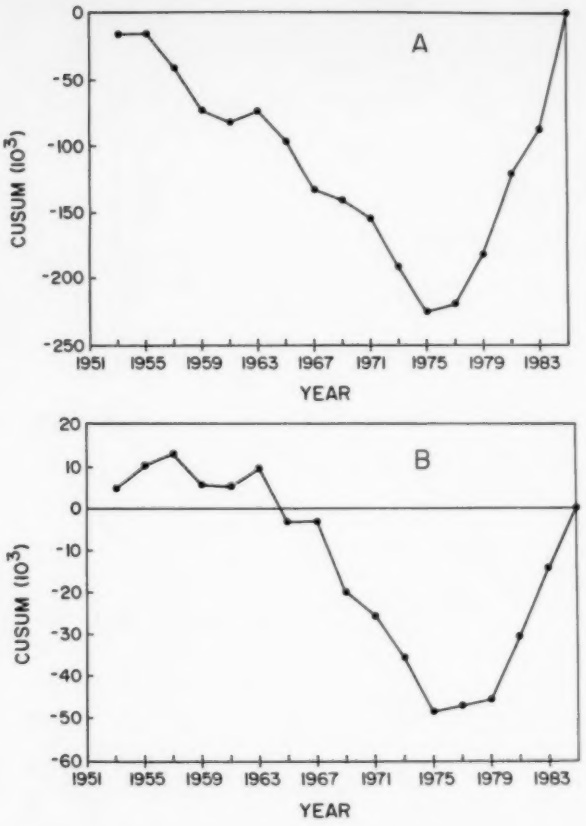

FIG. 17. A. Cumulative sum of the odd-year pink salmon catch (t) by (A) the United States, 1952–85, and (B) Canada, 1952–85 (INPFC 1979, 1977–89). Abundance trends in catch of both countries are similar from the early 1960s.

from table 1 in Eggers and Rogers 1987). Sockeye salmon production in Alaska continued to increase into the 1980s resulting in exceptional catches.

Pink Salmon (*Oncorhynchus gorbuscha*)

Pink salmon are the most abundant salmon in British Columbia and in the North Pacific Ocean. Eggs hatch in the spring following spawning and fry migrate almost immediately to sea. Pink salmon remain at sea for almost 18 mo when they return to their river of origin to spawn. Odd- and even-year spawning populations spawn in odd and even calendar years. In the south, pink salmon tend to spawn in odd-numbered years and in the north they tend to spawn in even-numbered years. In some rivers, such as the Fraser River, most spawners are almost exclusively of one type. Returns to the Fraser River in even years such as 1976 or 1978 are negligible. In the Skeena River the runs occur in both years, with the odd-year runs being slightly larger than the even-year runs. Because the runs remain genetically isolated, pink salmon catch trends need to be assessed by odd- and even-year spawning populations as well as total catch.

North American pink salmon catches increased in the late 1970s (Fig. 15A). Trends in catch (Fig. 15B) changed in 1977, approximately at the same time as those of sockeye salmon. The total catch increased from an average of 45.2×10^3 t in the period 1971–76 to an average of 126.4×10^3 t from 1977 to 1985. This catch pattern was strongly influenced by the United States catch that came mostly from Alaska. In the United States, the trend in Alaskan catches was most obvious in 1978 and 1979, the 1976 and 1977 brood years that went to sea in 1977 and 1978. In the United States, both odd- and even-year runs increased whereas in Canada, only the odd-year populations increased (Fig. 16). An increase in Canadian catches was apparent in 1977 and 1979, but most obvious in 1981, the 1979 brood year. The Canadian catch pattern was much more variable but shows a decline in the late 1960s and early 1970s similar to the United States and a recovery beginning in 1979.

If odd-year catches are examined separately, there was an increase in catch in both countries beginning in 1977 (Fig. 17). Again, the response was much larger in the United States in Alaska.

Even-year catches (Fig. 18A) were more variable than odd-year catches, and trends were different between Canada and the United States (Fig. 18B, 18C). The CuSum chart indicates that catch trends in the United States had four distinct trends from 1952 to 1984. From 1952 to 1960 the mean catch was 36.0×10^3 t, while from 1961 to 1968 the mean catch increased to an average of 60.6×10^3 t. Mean catch declined to 35.7×10^3 t from 1970 to 1976 and increased dramatically to 101.8×10^3 t beginning in 1978.

The catch trends off the west coast of Canada for even-year spawning stocks were similar except there was not an increase in average catch starting in 1978 (Fig. 18C). The decline in average catch that started about 1970 in both the United States and Canada continued in Canada through to 1984 (Fig. 18C).

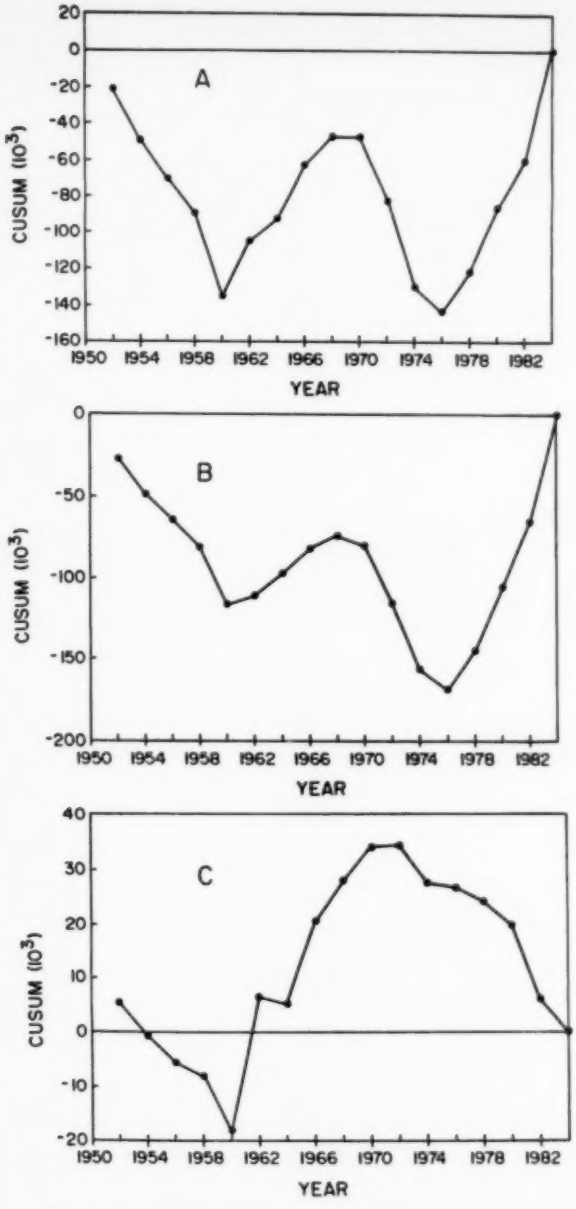

FIG. 18. (A) Cumulative sum of the even-year pink salmon catch (t) by Canada and the United States, 1952–85. The abundance trends are similar to the odd-year trends except during the 1960s. (B) Cumulative sum of the even-year pink salmon catch (t) by the United States, 1952–85. (C) Cumulative sum of the even-year pink salmon catch (t) by Canada, 1952–85. The abundance trends in the Canadian catch do not show the increasing trend in the late 1970s. (Data from INPFC 1979, 1977–89.)

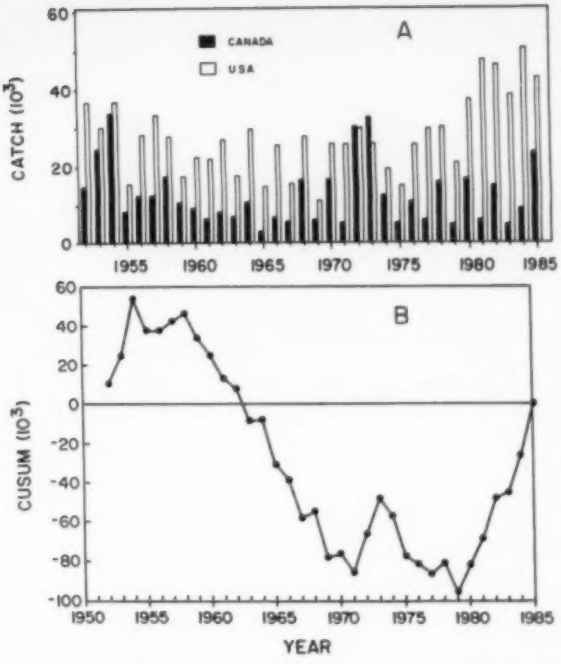

FIG. 19. (A) Chum salmon catch (t) by country and (B) cumulative sum of the chum salmon for the combined catch (t) by Canada and the United States, 1952–85 (INPFC 1979, 1977–89).

Chum Salmon (*Oncorhynchus keta*)

Chum salmon are the latest of the salmon to spawn, although some may enter streams in early summer and spawn in summer. After hatching and emerging from the gravel in the spring, the fry migrate directly to sea. Juvenile chum salmon have the widest natural distribution of all Pacific salmon species (Fredin et al. 1977).

The highest percentage of maturing adults return after spending 3 yr at sea or 4 yr after eggs are fertilized. Adults can range in age from 2 to 7 yr (Salo 1991), but most are 3-5 yr old (from spawning) when they return to spawn. Thus, most of the fish that returned in 1980 as 4-yr-olds were spawned in 1976 and went to sea in 1977.

Annual catches in the United States were about double the Canadian catches in most years (Fig. 19A). The mean total catch from both countries from 1958 to 1971 was 31.5×10^3 t without large fluctuations in the CuSum chart (Fig. 19B). There was an increase in the mean catch in 1972 and 1973, followed by a decrease in the mean from 1974 to 1979 (Fig. 19B). The trend in the mean catch increased abruptly in 1980. The shape of the CuSum chart for catches in the United States was similar to that for sockeye and pink salmon catches in the United States fishery. In the Canadian fishery, catch trends were similar to the United States fishery up to 1971; there was an increase in catch from 1972 to 1973 and a decline from 1974 to 1984. There was no increase in mean catch beginning in 1980 as was observed in the United States fishery.

Chum salmon returning in 1980 were the 1976 brood year that went to sea in 1977. The change in catch trends that occurred in the Alaskan fishery in 1980 is another indication that, in general, 1977 was a year of above-average marine survival for salmon. The absence of a response by Canadian stocks may indicate that improved survival of chum salmon occurred initially in the more northern areas of their distribution.

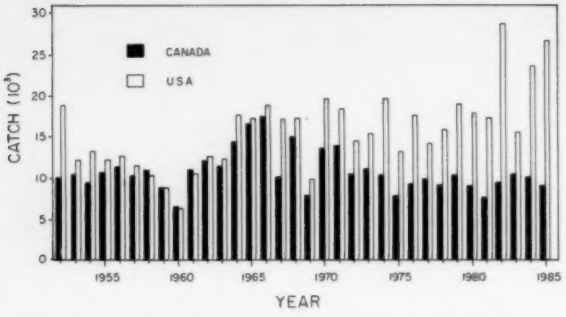

FIG. 20. Coho salmon (t) by country (INPFC 1979, 1977–89).

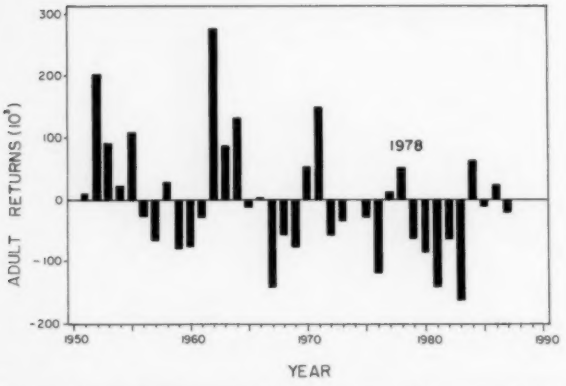

FIG. 21. Anomalies from the long-term mean of returns of wild-spawning coho salmon to the Fraser River (data courtesy of K. Wilson and R. Kadowaki, Pacific Biological Station, Nanaimo, B.C.).

Coho Salmon (*Oncorhynchus kisutch*)

Coho salmon are distributed along the coast of North America from California to Alaska and into the Bering Sea (Hart 1973). Eggs are deposited in the fall and hatch the following spring, with most fry remaining in streams for 1 yr before entering the sea. Juveniles remain at sea for an average of 18 mo. Most coho salmon are therefore age 3 when they spawn and die in fresh water.

Catches of coho salmon in the Canadian and United States fisheries from 1952 to 1985 (Fig. 20) were similar until 1970, after which United States catches increased and Canadian catches declined slightly. Canada and the United States have extensive enhancement programs that contributed an increasing number of coho salmon to the fishery beginning in the mid-1960s in the United States and mid-1970s in Canada. Because of the complexity of separating hatchery catches from wild catches and of interpreting the impact of the hatchery program on wild coho salmon population dynamics, I have not used total catches of coho salmon to examine trends in abundance. Instead, I looked at returns from some of the major Canadian coho salmon fisheries.

In British Columbia, more coho salmon are produced in the Fraser River than in any other system. Coho salmon that went to sea in 1977 were from the 1975 spawning or brood year and returned to spawn in 1978. The total returns (catch plus spawners) from the 1975 brood year were above average relative to the average returns since 1951 (Fig. 21). Except for the returns from the 1981 brood year, the returns from coho that went to sea

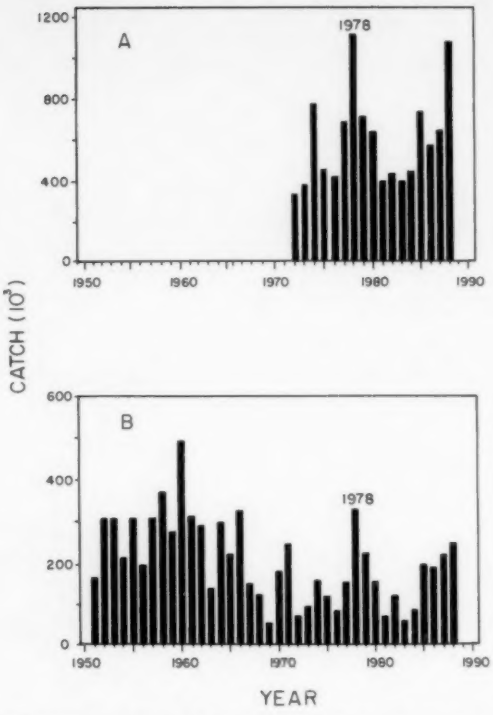

FIG. 22. (A) Sports catch and (B) troll catch of coho salmon in the Strait of Georgia (data courtesy of K. Wilson and R. Kadowaki, Pacific Biological Station, Nanaimo, B.C.).

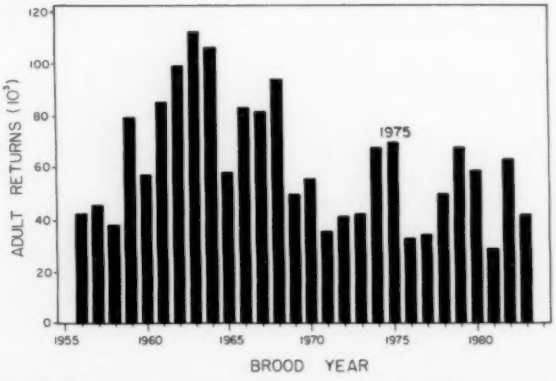

FIG. 23. Total return (catch + escapement) of age 3 coho salmon to the Skeena River (from Kadowaki 1988), by brood year. Most fish from the 1975 brood year went to sea in 1977.

in 1977 were the highest wild returns over the period 1971–87. The returns from the 1981 brood year were similar to the returns from the 1975 brood year.

The increase in survival of the 1975 brood year is also reflected in the coho salmon catch in the sports fishery and the commercial troll fishery in the Strait of Georgia in 1978. The sports catch in 1978 was the highest over the period 1972–88 (Fig. 22A). Similarly, the commercial troll catch was the highest on record over the period 1967–88 (Fig. 22B).

In the Skeena River, coho salmon spend 1 or 2 yr in fresh water

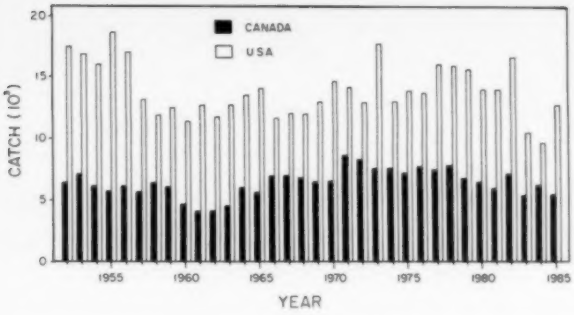

FIG. 24. Chinook salmon catch (t) by country (INPFC 1979, 1977–89).

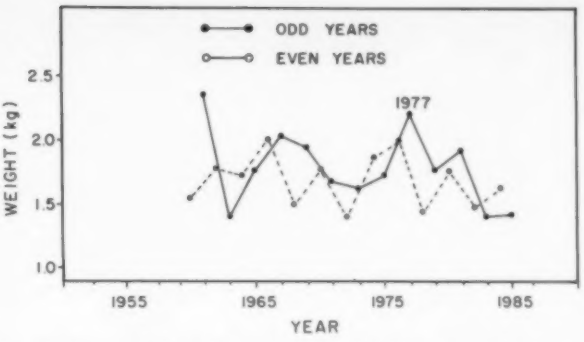

FIG. 26. Average weight of pink salmon caught in the fishery in southeast Alaska (data courtesy of W.E. Ricker, Nanaimo, B.C.). The trend of declining size changed briefly beginning in 1977.

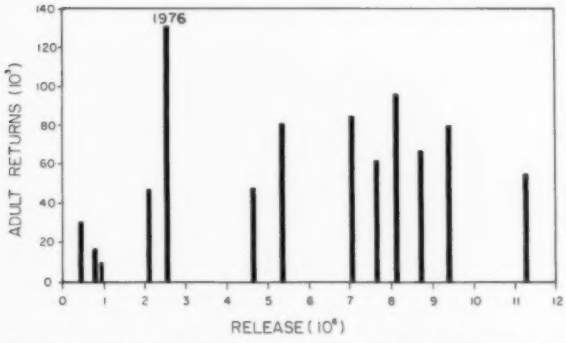

FIG. 25. Releases and returns of chinook salmon from hatcheries in the Strait of Georgia, 1972–85 (data from Beamish et al. 1992). Releases are recorded for brood years. The fish from the 1976 brood year went to sea in 1977.

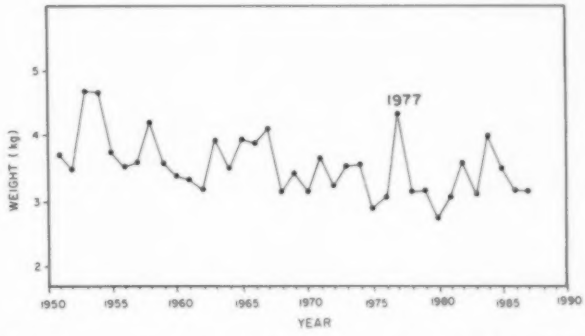

FIG. 27. Average weight of coho salmon caught by the troll fishery in the northern coast off the Queen Charlotte Islands, B.C. (data courtesy of W.E. Ricker, Nanaimo, B.C.).

after hatching, returning to spawn in their third or fourth year after 18 mo in the marine environment. The dominant age class on return are 3-yr-old fish that have spent 2 yr in fresh water. Consequently, coho that went to sea in 1977 and returned in 1978 would be predominantly the 1975 brood year with a smaller component from the 1974 brood year. The total returns from the 1975 and 1974 brood years were above average for the returns of all brood years over the period 1969–83 (Fig. 23).

Chinook Salmon (*Oncorhynchus tshawytscha*)

Chinook salmon have the most complicated life history of the five salmon species found in British Columbia. In general, there are two life history types, the ocean type and the stream type. The ocean-type fish spawn in the fall and go to sea the following spring. Chinook salmon raised in hatcheries are all ocean type. Stream type spawn in the fall and remain in the spawning stream for 1 yr. When returning to spawn, stream type generally arrive earlier than the ocean type. Most chinook salmon return to spawn from age 3 to 5 (Healey 1991).

Catches of chinook salmon in the United States and Canadian fisheries were more constant than the catches of other salmon from 1952 to the early 1980s (Fig. 24), with catches in the United States always larger than Canadian catches (Fig. 24). Chinook salmon catches, like coho salmon catches, were influenced by hatchery programs. Catch information, therefore, is not necessarily a good indicator of abundance trends. Also, because of the complex life cycle of the chinook salmon, the very limited amount of age information available, and the complexities of the fisheries, it is difficult to study the relative success of year classes. It is possible, however, to examine the survival of chinook salmon that were released from hatcheries. Hatchery releases in Canada indicate that chinook salmon that went to sea in 1977 had increased survival. The returns from hatcheries that released smolts into the Strait of Georgia were the highest in the 1977 release year even though the number of smolts released increased after 1977 (Fig. 25).

Growth of Maturing Salmon during 1977

The average size of pink and coho salmon has declined since 1950 (Ricker et al. 1978; Ricker and Wickett 1980). There were annual fluctuations in the average size of fish caught in the various fisheries, but there was no doubt that a decrease in mean size occurred. The analyses have recently been extended (W.E. Ricker, Pacific Biological Station, Nanaimo, B.C., unpublished data) to the late 1980s. In many fisheries, the decline in average weight continued. However, in 1977 there was clear evidence that the size of salmon in the commercial catches was anomalously large for pink, coho, and chinook salmon. For pink salmon the increase in size was particularly evident for the odd-year spawning stocks in southeastern Alaska and northern British Columbia (Fig. 26). Because pink salmon grow most during their second year in salt water, it is evident that feeding conditions

TABLE 1. Landings from the major fisheries off the west coast of Canada and the United States for 1988. The landings for salmon are the average annual landings from 1983 to 1986. The category of others includes rockfish, flatfish, and other "minor" species. n/a, not applicable because no fishery exists.

Species	Canadian landings (t)	U.S. landings (t)	Total (t)
Pink salmon	24 567	116 502	141 069
Sockeye salmon	23 962	108 921	132 883
Chum salmon	15 570	45 469	61 039
Coho salmon	10 438	24 915	35 353
Chinook salmon	5 855	12 916	18 771
Pacific herring	30 998	67 533	98 531
Pacific halibut	4 762	26 850	31 612
Walleye pollock	577	1 283 423	1 284 000
Pacific cod	10 835	228 665	239 500
Pacific hake	90 491	170 688	261 179
Northern anchovy[a]	n/a	84 887	84 887
Chub mackerel	n/a	54 671	54 671
Sablefish	5 196	43 854	49 050
Pacific ocean perch	6 673	16 124	22 797
Lingcod	2 508	2 976	5 484
Yellowtail rockfish	4 778	6 263	11 041
Widow rockfish	2 065	10 891	12 956
Atka mackerel	n/a	30 061	30 061
Others	19 534	295 887	315 421
Total	258 809	2 631 496	2 890 305

[a]Includes catches off Mexico.

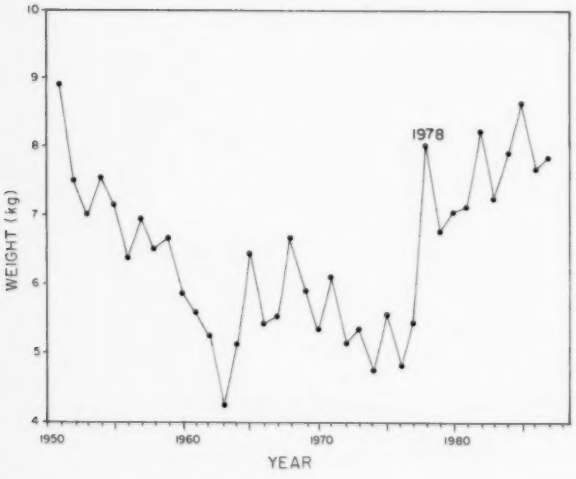

FIG. 28. Average weight of chinook salmon caught in the Kitimat-Butedale area troll fishery (data courtesy of W.E. Ricker, Nanaimo, B.C.). The trend of declining sizes changed abruptly in 1978.

were above average in 1977. Most coho salmon also return to spawn after their second year in salt water. It is during this second year, the year in which they are captured by the commercial fishery, that they increase most in weight. In 1977, in 33 of 42 fisheries, coho salmon were much larger than in adjacent years (Fig. 27). These increases in size occurred mostly in the north and not in many of the southern areas or in Puget Sound.

W.E. Ricker (unpublished data) also identified 1978 as a year that produced large chinook salmon. Because chinook salmon are taken by troll over a much longer season than pink or coho salmon, they will have undergone most of their growth in the calendar year before capture in 1977 (Fig. 28). W.E. Ricker (unpublished data) identified a decreasing trend in average sizes of chinook salmon from 1951 to 1975 in 18 of 19 areas from southeast Alaska to Puget Sound. Starting in 1977 or 1978, the trend changed and the average size increased (Fig. 28 shows one of the more extreme changes in trend). This change first appeared in northern stocks, with some southern stocks showing the increasing trend by about 1983. Some southern stocks have not increased in average size.

W.E. Ricker (unpublished data) identified nine possible causes of the trends in size changes, including a change in the ocean environment that would affect growth rate, age at maturity, or both. The cause of the growth rate changes remains a mystery, but the synchronous nature of the trends in the late 1970s indicates both an association with the ocean environment and that food for these salmon species was abundant in 1977. The increased growth and the increased survival indicate that there must have been an increase in marine carrying capacity for these species of salmon in 1977.

Nonsalmon Fisheries

Changes in the abundance of nonsalmon fisheries were examined for fishes that contributed to commercial fisheries and had a reliable time series of catch or other biological information beginning before the mid-1970s. The following accounts of strong year-class production exclude examples of fisheries that did not show increases in productivity in the late 1970s, but these fisheries, identified as "others" in Table 1, represented only a small percentage (11%) of the total catch (Table 1). I used published data to develop indices of year-class strength and combined these indices using the method described by Ebbesmeyer et al. (1991) to show that there was a synchronous increase in year-class survival from 1976 to 1978. In the following species

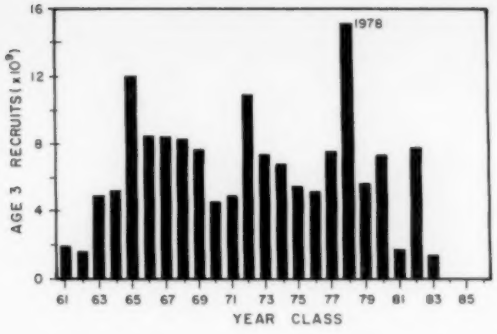

FIG. 29. Calculated number of age 3 walleye pollock in the eastern Bering Sea using surface otolith age estimates (from Wespestad and Traynor 1987).

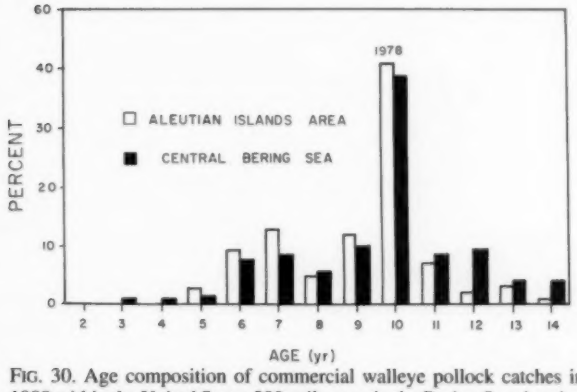

FIG. 30. Age composition of commercial walleye pollock catches in 1988 within the United States 200-mile zone in the Bering Sea showing the strong 1978 year class; ages estimated from otolith surfaces (from Dawson 1988).

summaries, I briefly describe the biology and present relevant fisheries information to show the extent and diversity of the response. At the end of the species accounts, I combine the year-class strength indices to show that there was a synchronous increase in survival for most species about 1977.

Walleye Pollock (*Theragra chalcogramma*)

Walleye pollock spawn in the midwater or just off bottom from late February until late May. Most spawning occurs in early April. There may be more than one spawning event in some areas, resulting in several pulses of spawning (Bulatov 1988; Haldorson et al. 1988). The pelagic larval walleye pollock move from near the bottom after hatching to a depth of about 50 m (Kim 1988). At this time, copepod nauplii are the most important food for larvae smaller than 14 mm (Kendall et al. 1987). Larger larvae concentrate near the surface in late May and feed on adult copepods (Kendall et al. 1987). In Auke Bay, Alaska, maximum abundance of larval walleye pollock in the surface waters occurred at the time of maximum abundance of herbivorous copepods (Haldorson et al. 1988). Age 2 and age 3 fish are taken in the fishery, but full recruitment is not considered to occur until age 4.

A reconstruction of year-class strength, using age estimates from otolith surfaces, identified the 1978 year-class as the

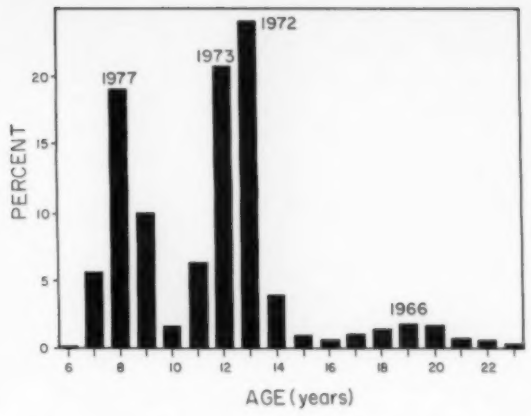

FIG. 31. Age composition of walleye pollock sampled in February 1985 by Polish scientists in the international area of the Bering Sea ($n = 299$). Abundance of age groups was determined using an age–length key to convert length–frequency distributions to age–frequency distributions. Relative year class strength is in percent. Ages determined using the method of McFarlane and Beamish (1990).

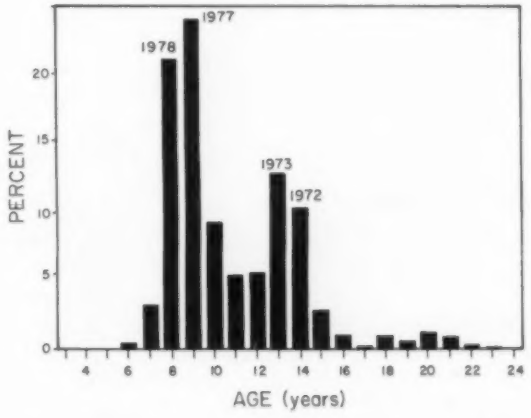

FIG. 32. Age composition of walleye pollock sampled from January to April 1986 by Polish scientists in the international area of the Bering Sea ($n = 1600$). Abundance of age groups was determined using an age–length key to convert length–frequency distributions into age–frequency distributions. Relative year class strength is in percent. Ages determined using the method of McFarlane and Beamish (1990).

strongest on record in the Bering Sea (Fig. 29), and it still dominates the United States commercial catch (Fig. 30). In the western Bering Sea, both the 1977 and 1978 year classes were thought to be very large (Moiseyev 1983). It is possible that both year classes were strong, but it is also probable that errors in determining age resulted in fish from one strong year class being assigned to other age classes. A review of possible aging errors (McFarlane and Beamish 1990) indicated that ages determined from examinations of the otolith surface could underestimate the actual age of some stocks of walleye pollock. The new ages produced by the method suggested by McFarlane and Beamish (1990) indicated that more age groups existed in samples from commercial fisheries than previously thought.

The new interpretation of age was used to estimate the age of

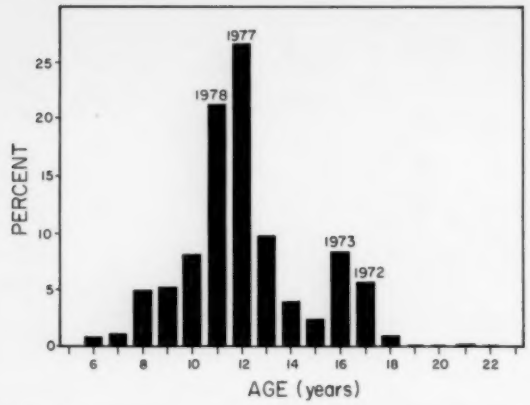

FIG. 33. Age composition of walleye pollock sampled from January to April 1989 by Polish scientists in the international area of the Bering Sea (n = 1201). Abundance of age groups was determined using an age-length key to convert length-frequency distributions to age-frequency distributions. Relative year class strength is in percent. Ages determined using the method of McFarlane and Beamish (1990).

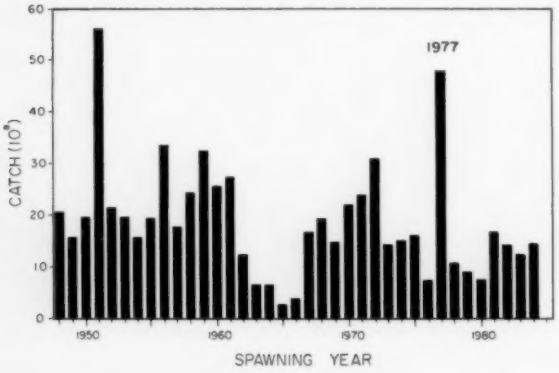

FIG. 34. Calculated number of age 2+ Pacific herring in the population by spawning year (Haist et al. 1988).

walleye pollock caught in the Polish commercial fishery in the Bering Sea in 1985 (data provided by Dr. J.F. Janusz, Sea Fisheries Institute, Gdynia, Poland). Because this sample was obtained at the beginning of the fishery, it represents the age composition of a relatively unexploited population. In 1985, 299 fish were aged from a sample collected from January to April (Fig. 31) and 1600 fish were aged from a sample collected in the same period in 1986 (Fig. 32). Both the 1985 and 1986 samples indicated that very strong year classes occurred around 1965 and 1966, 1972 and 1973, and 1977 and 1978. The age composition of the 1989 Polish catch (Fig. 33) shows that the 1977 and 1978 year classes are still important, indicating that an exceptionally strong year class or year classes occurred about 1977. Because the walleye pollock fishery is extremely large, the exceptionally strong year classes produced around 1977 and 1978 represent a large increase in fish biomass.

Pacific Herring (*Clupea pallasi*)

The Pacific herring fishery is one of the oldest fisheries in British Columbia and was also the largest in British Columbia

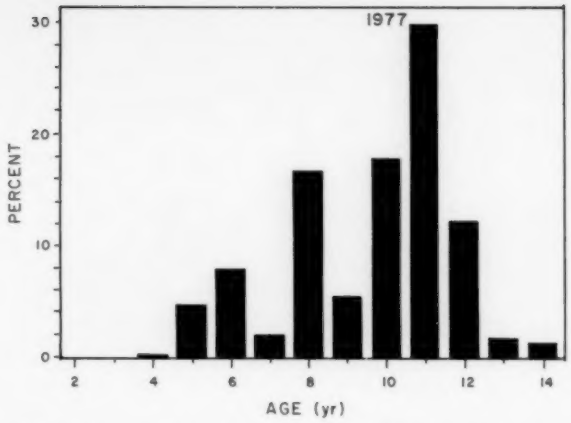

FIG. 35. Age composition of the 1988 catch of Togiak District Pacific herring showing the strong 1977 year class (from Funk and Savikko 1989).

from 1955 to 1967. The fishery shut down in the late 1960s and resumed again in the early 1970s. Currently the fishery harvests about 35 000 t for a roe market. Herring catches off the coast of Alaska are currently larger than off the coast of British Columbia, but historically, catches in the herring fishery off the coast of Alaska were less than one half of those in British Columbia. Most herring in the Alaska fishery range from 4 to 12 yr and in British Columbia from 2 to 5 yr.

The relative abundance of age 2+ herring in the Canadian fishery was estimated from 1948 to 1984 using an age-structured model (Haist et al. 1988). The abundance of age 2 herring from the 1977 spawning was the second strongest year class on record (Fig. 34). Off the Alaskan coast, strong year classes in the five largest stocks, representing 90% of the catch, were produced in 1977 (Funk and Savikko 1989). Strong year classes were also reported in some Alaskan stocks in 1976 and 1978. In the Togiak stock in Alaska the strong 1977 and 1978 year classes still dominated catches in 1988 (Fig. 35).

Pacific Cod (*Gadus macrocephalus*)

The centre of abundance of Pacific cod is off the coast of Alaska, in the eastern Bering Sea. In British Columbia, they are close to the southern limit of their commercial abundance. Pacific cod grow rapidly and are fully recruited into the fishery by age 3. Few fish survive to age 7 in the Canadian fishery. Older fish occur in the fishery off Alaska; however, stock assessment models assume a maximum age of 8 yr in the catch (Zenger and Thompson 1989a). Zenger and Thompson (1989a) estimated that from 1975 to 1985, in the eastern Bering Sea, the 1977 year class had exceptional production as indicated by age 3 individuals (Fig. 36). A strong 1976 year class was also identified. However, Pacific cod can be difficult to age (Beamish et al. 1990), making it possible that aging error contributed to the appearance that both the 1976 and 1977 year classes were strong.

In the Gulf of Alaska, the 1977 year classes dominated the fisheries in the early 1980s (Zenger and Thompson 1989b). Zenger and Thompson (1989b) did not provide the age composition of the catch because it "has proven to be a frustrating task." However, using age 3 as the age of recruitment, it appears that the more than doubling of the catch in 1980 compared with 1978 and 1979 is the result of a strong 1977 year class.

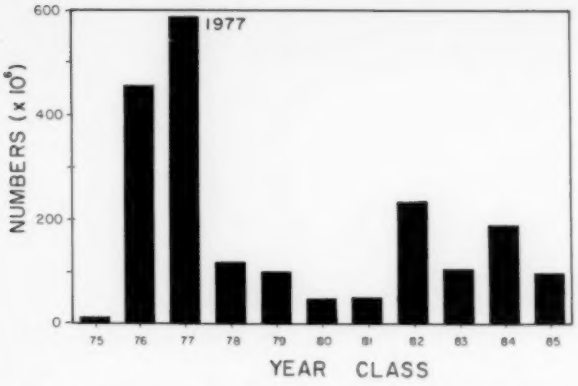

FIG. 36. Estimated number of age 3 Pacific cod recruited into the fishery in the eastern Bering Sea from 1975 to 1985 (from Zenger and Thompson 1989a).

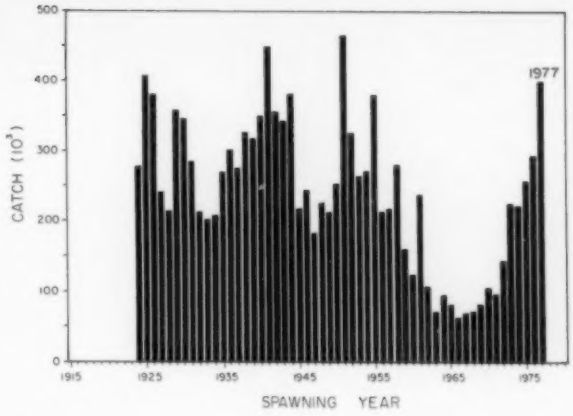

FIG. 37. Calculated number of age 11 Pacific halibut in the commercial catch (data from the International Pacific Halibut Commission).

Age estimates have not been determined for the Canadian commercial fishery for Pacific cod in the late 1970s and early 1980s. However, an index of the size of the Hecate Strait population indicated that a significant increase in survival occurred in 1977 (Tyler and Westrheim 1986).

Pacific Halibut (*Hippoglossus stenolepis*)

Pacific halibut is one of the most important commercial bottom fish off the west coast of North America. The centre of abundance of Pacific halibut is in the Gulf of Alaska and in the northern waters off the coast of British Columbia. Reliable estimates of the age composition of the catch in the commercial fishery exist from 1923, making this data series one of the longest of all west coast fisheries. Pacific halibut are first recruited to the fishery at age 8 and fully recruited by age 11. The International Pacific Halibut Commission publishes the age composition of commercial catches. In 1988, the catch consisted of a large number of age 11 fish, from the 1977 year class. When the catch of age 11 fish is compared with the catches of similar aged fish, it is apparent that the 1977 year class was exceptionally strong (Fig. 37). The 1977 year class remained the dominant age class in the fishery into the early 1990s (International Pacific Halibut Commission, Seattle, WA 98145-2009, unpublished data). Beginning in 1986, recruitment of 8-yr-olds declined (International Pacific Halibut Commission, unpublished data), indicating that year class strength declined after 1977.

Pacific Ocean Perch (*Sebastes alutus*)

Pacific ocean perch was historically the most important species of rockfish in the Canadian and United States fisheries. Pacific ocean perch give birth along the coast from California to the eastern Bering Sea from January to March. They are long-lived and are not recruited into the fishery until age 8–11. As a result of extensive foreign fishing for Pacific ocean perch off the west coast of North America from the mid-1960s until the late 1970s, catches declined dramatically in this period. In the Gulf of Alaska, for example, catches were as high as 350 000 t in 1965 but declined to about 5500 t after 1977 (Balsiger et al. 1985). Reliable age estimates are available from the late 1970s. Prior to this, age determinations seriously underestimated the actual age of many fish caught in the fishery (Beamish 1979). Strong year classes were produced off the coast of Canada, in

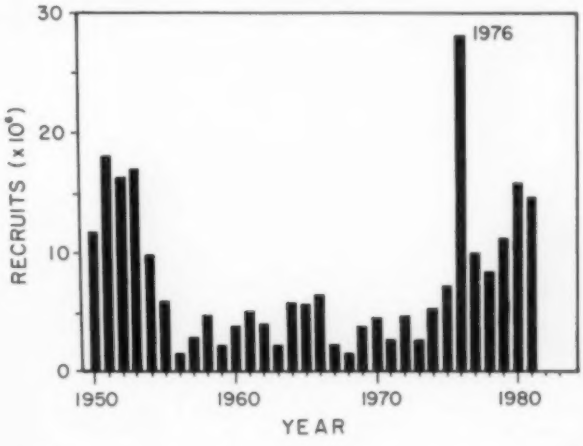

FIG. 38. Estimate of age 9 Pacific ocean perch in the Gulf of Alaska population (Heifetz and Clausen 1992; data from J. Heifetz, Auke Bay Laboratory, Juneau, Alaska).

the Gulf of Alaska (Fig. 38), and in the Bering Sea in 1976 (Fargo and Tyler 1989; Ito 1989; Ronholt 1989; Heifetz and Clausen 1992). It is possible that 1976 may not be the correct year of the strong year class because of aging errors, but a strong year class clearly was produced about this time.

Yellowtail Rockfish (*Sebastes flavidus*)

Yellowtail rockfish are most abundant off the coasts of southern British Columbia, Washington, and Oregon. Yellowtail rockfish are slow growing and long-lived; the oldest recorded age is 64 yr (Chilton and Beamish 1982). They are first captured at about age 5 and are fully recruited at age 14 (Tagart 1988). In Canada, the total landings in 1988 were 5100 t, including 334 t of incidental catch in the offshore Pacific hake (*Merluccius productus*) fishery. This was the second largest rockfish fishery, accounting for 18.5% of the total rockfish catch. In the United States, 1988 landings totalled 6263 t, of which 89% was from waters off the coasts of Washington, Oregon, and northern California.

In 1986, 9-yr-olds (the 1977 year class) dominated the age groups in the Canadian catch. In 1988, the 1977 year class still

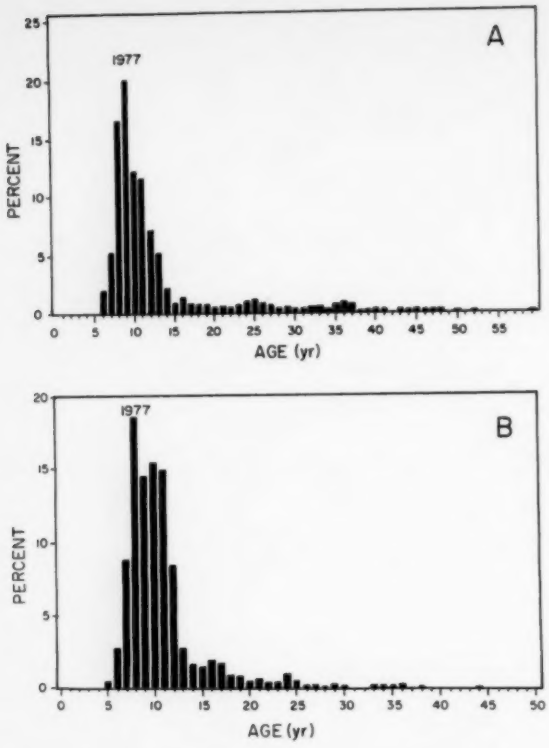

FIG. 39. (A) Age composition of yellowtail rockfish caught in Queen Charlotte Sound in 1986 showing the strong 1977 year class (data courtesy of R. Stanley, Pacific Biological Station, Nanaimo, B.C.) and (B) estimated yellowtail rockfish in the catch by age off Washington in 1985 (from Tagart 1988).

continued to dominate age groups in the catch (Fig. 39A). In the United States, the strong 1977 year class appeared in catches off the coast of Washington (Fig. 39B) but not farther south (Tagart 1988).

Widow Rockfish (*Sebastes entomelas*)

Widow rockfish is a valuable species in the rockfish catch off the coasts of Washington, Oregon, and California. In 1987, these catches totalled 12 185 t (Lenarz and Hightower 1988). In 1988, catches off the west coast of Canada were 2065 t. Because they are long-lived, with a maximum age of 58 yr (Chilton and Beamish 1982), the age at full recruitment is probably 8–11 yr, similar to other long-lived rockfish species. The age at first capture is 4 yr; thus the 1977 year class would not appear in the catches until 1981. Lenarz and Hightower (1988) estimated the total number of widow rockfish for each year class caught by the fishery from 1980 to 1987. In 1984, the 1977 and 1978 year classes dominated the catch (Fig. 40). The estimated age composition of the 1987 catch indicated that the 1977 and 1978 year classes were still strong and that the production of widow rockfish increased from 1977 into the 1980s (Lenarz and Hightower 1988).

Atka Mackerel (*Pleurogrammus monopterygius*)

Atka mackerel are found along the continental slope off the United States and Russia in the northern North Pacific Ocean

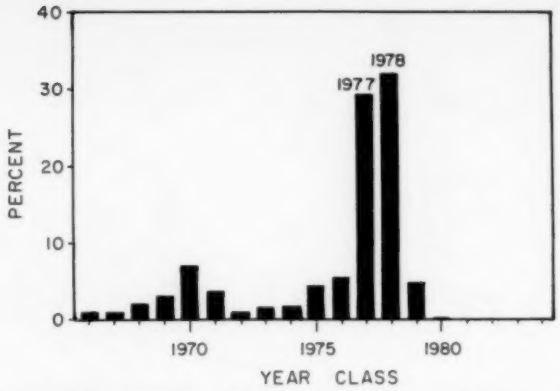

FIG. 40. Widow rockfish catch by year class in 1984 (from Lenarz and Hightower 1988). Year classes prior to 1966 were not included because of their low relative abundance.

and the Bering Sea, with the centre of abundance along the Aleutian Islands. Age at recruitment is 3 yr (McDevitt 1989) and most fish in the fishery are less than 13 yr old (McDevitt 1989). Catches within the United States 200-mile limit were at record levels from 1984 to 1987, averaging 34 000 t. The catch dropped slightly in 1988 to 30 061 t.

The strong 1977 year class first appeared in the catch in 1980 at age 3, accounting for 52% of the age classes in the catch (Fig. 41A). The 1977 year class dominated the catch in 1982 when it was 78% of the numbers landed and 69% of the total weight (Fig. 41A). In 1987, even though a strong 1984 year class entered the fishery, the 1977 year class represented 17% of the catch by number and 21% of the catch by weight (Fig. 41B).

Sablefish (*Anoplopoma fimbria*)

The sablefish fishery occurs off the coasts of California, Oregon, and Washington and is most important off the coast of British Columbia and in the Gulf of Alaska. In recent years, this fishery in Canada has become the most valuable Canadian groundfish fishery. Sablefish spawn in mid-February and are long-lived (Beamish and Chilton 1982). They are recruited to the fishery about age 5 and can live for over 60 yr. Recent age estimates and length frequency distributions indicate that an exceptionally strong year class occurred in 1977 throughout most of the spawning range (McFarlane and Beamish 1983; Sigler and Fujioka 1988). The 1977 year class was so strong that it dominated the age composition of catches well into the 1980s (Fig. 42). Other strong year classes occurred in the late 1950s and early 1940s (McFarlane and Beamish 1992). Strong year classes in general and the 1977 year class in particular were recently shown to be significantly related to coastwide increases in copepod production (McFarlane and Beamish 1992).

Pacific Hake (*Merluccius productus*)

Pacific hake occur off the coasts of California, Oregon, Washington, and British Columbia. The Canadian Pacific hake fishery lands the largest catch, by weight, of any species in the Canadian fishery. Pacific hake caught in the fishery off Canada are part of an offshore population that spawns off the coasts of California and Mexico, mainly during January and February (Alverson and Larkins 1969). This offshore population has been fished commercially for approximately 25 yr. Current abundance

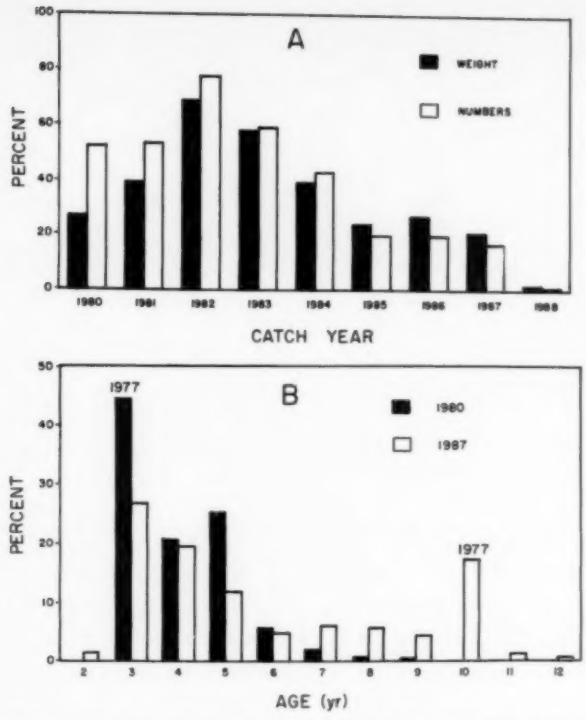

FIG. 41. (A) Percent by number and weight of the 1977 year class of Atke mackerel in catches from 1980 to 1988 (from McDevitt 1989) and (B) estimated age of catch of Atka mackerel in the Aleutian Islands region for 1980 and 1987 (from McDevitt 1989).

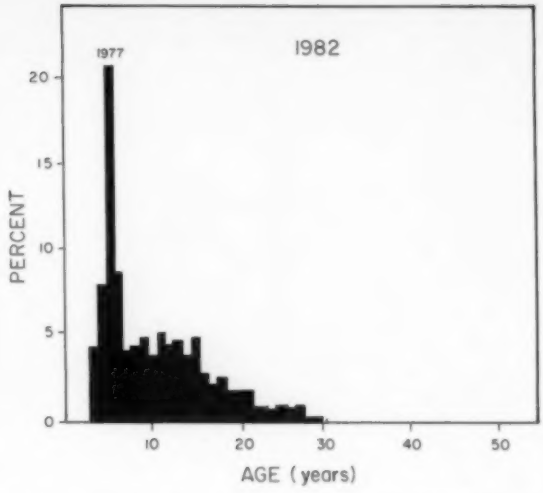

FIG. 42. Age composition of the commercial catch of sablefish in 1982 from the west coast of Canada (from Fargo et al. 1988).

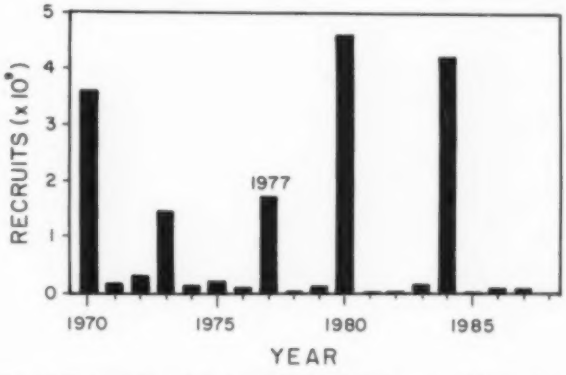

FIG. 43. Estimated number of age 2 Pacific hake determined from cohort analysis that would be recruited into the fishery from each of the year classes (from Dorn and Methot 1989).

estimates vary but appear to average about 1 250 000 t (Francis 1983).

Eggs and newly hatched larvae are found at depths of 40–100 m. Larvae feed on copepod eggs and nauplii, and adults feed heavily on euphausiids. Juveniles mature at age 3. They are recruited into the fishery from age 3 to age 6. Older and larger fish migrate into the Canadian zone in late spring and early summer and return to spawn in the fall. Because mature females are larger than mature males, more females are captured in the fishery off Canada than males.

The presence of strong year classes is a common feature of the offshore population (Bailey and Francis 1985). Dorn and Methot (1989) calculated that strong year classes occurred in the fishery in 1970, 1973, 1977, 1980, and 1984 (Fig. 43). These strong year classes dominated the age composition of the catch in the fishery off Canada. Although the 1977 year class was not as strong as some others, it was still abundant in the Canadian fishery in the late 1980s (Fargo et al. 1988).

Chub Mackerel (*Scomber japonicus*)

Most chub mackerel occur off the coast of California where spawning occurs inshore at depths to 92 m from late April to July (MacCall and Prager 1988). Females may spawn more than once during this period. The maximum age is 9 or 10 yr, but most fish are fully recruited into the fishery at age 4 (MacCall et al. 1985). The chub mackerel fishery in the United States started in 1929, but collapsed in the late 1960s, and in 1970 the California fishery was closed (Parrish and MacCall 1978). It reopened in 1977 when abundance appeared to be increasing. Total United States and Mexico catches increased from 4064 t in 1976–77 to 42 361 t in 1981–82 (MacCall et al. 1985).

Chub mackerel abundance from 1977 to 1984 was the highest in almost 50 yr (MacCall et al. 1985). The 1976 year class was produced from a very small population of adults, but the strongest year class was produced in 1978 (Fig. 44). This year class was the largest on record 27–52% larger than the previous record, the 1932 year class. Three of the four largest year classes in the history of the fishery were produced in 1978, 1980, and 1981 (Fig. 44).

Lingcod (*Ophiodon elongatus*)

The fishery for lingcod is one of the oldest in British Columbia (Cass et al. 1990). Most lingcod are recruited to the fishery between age 5 and age 6. Maximum age is believed to be 21 yr (Chilton and Beamish 1982). Because a method to determine the age of lingcod was only recently developed (Beamish and Chilton 1977; Cass and Beamish 1983), there are relatively few

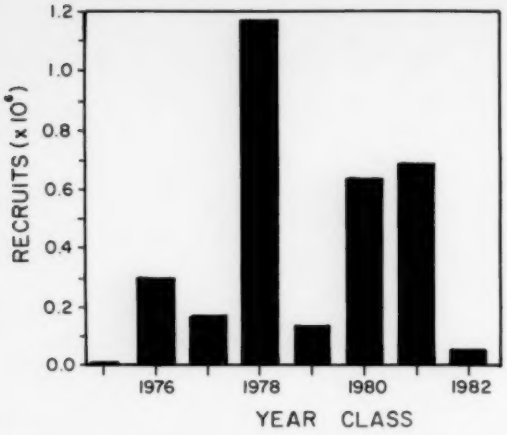

FIG. 44. Annual estimated catches of age 1 chub mackerel from 1976 to 1982 shown for each year class (MacCall et al. 1985). The strong 1978 year class was produced from a very small population of adults.

TABLE 2. Age estimates from trawl catches of lingcod.

	Number of fish	
Age (yr)	1982	1983
3	5	2
4	27	47
5	42	152
6	47	148
7	35	74
8	14	30
9	3	12
10	6	7
11	1	4
12	1	6
13		3
14		3
15		—
16		1

estimates of age composition. Catch can be used, however, to identify changes in population size. Most lingcod in the commercial catch are age 5 and age 6 (Table 2). The 1977 year-class would recruit into the catch in 1982 and 1983, and the total lingcod catch from all areas in Canadian waters in these years was higher than in the previous 11 yr (Fig. 45; Tyler and Fargo 1990). Without adequate estimates of the age composition of the catch, it is difficult to identify the exact year or years when strong year classes were produced. However, it is probable that 1976 was not a strong year class because 1981 was not a year of high catch and it is likely that lingcod would be recruited at age 5. Thus, it is probable that 1977 was a year that produced a strong year class that contributed to the increased catches beginning in 1982.

Northern Anchovy (*Engraulis mordax*)

Northern anchovy are fished off the coasts of the United States and Mexico. The United States fishery started in 1954 with landings of 25 300 t. The Mexican fishery started in 1964.

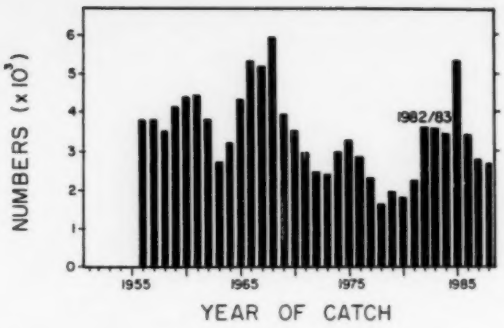

FIG. 45. Canadian catch of lingcod off the west coast of British Columbia, 1956–89.

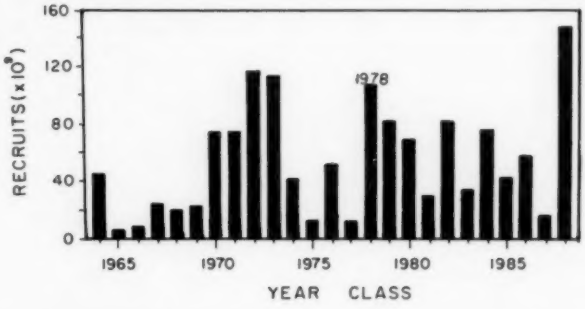

FIG. 46. Estimated abundance of age 0 northern anchovy on July 1 recruited to the fishery from 1966 to 1988 shown for each year class (data estimated from Jacobson and Lo 1989).

Recruitment to the fishery is considered to occur at a nominal age of 0.5 yr. Before the mid-1970s, the largest combined catch of 141 400 t occurred in 1973. In 1976, the year class was strong and the 1977 year class was the fourth lowest in 31 yr (Methot 1989). In 1978, recruitment was the fourth highest in 31 yr, despite the low spawning biomass. The fishery off the United States coast declined to a very low level in 1983. The fishery off the Mexican coast continued, primarily on age 0 fish. The estimated recruitment time series (Jacobson and Lo 1989) shows the strong year class in 1978 that followed a series of weak year classes (Fig. 46).

Trends in Nonsalmon Recruitment Indices

The data used to identify strong year classes for nonsalmon fishes can be combined using the procedure described by Ebbesmeyer et al. (1991). The year class strength data was converted to a nondimensional anomali index according to the equation

$$[\ln R - (\Sigma \ln R/n)]/\mathrm{Sd}\,(\ln R)$$

where R is the index of relative year class strength. The relative year class strength indices used were as follows: walleye pollock, 1986 sample collected in the Bering Sea and converted to show relative year class strength (Fig. 32); Pacific herring, the relative abundance of age 2+ herring in the Canadian fishery as estimated by Haist et al. (1988) (Fig. 34); Pacific cod, the number

of age 3 fish recruited into the fishery from 1975 to 1985 as estimated by Zenger and Thompson (1989a) (Fig. 36); Pacific ocean perch, the estimated age 9 fish in the Gulf of Alaska population as estimated by Heifetz and Clausen (1992) (Fig. 38); yellowtail rockfish, data used to reconstruct relative year class strengths from a recent unpublished report by R.D. Stanley, Pacific Biological Station, Nanaimo, B.C.; widow rockfish, the number of fish in the catch for each year class in the 1984 fishery as estimated by Lenarz and Hightower (1988) (Fig. 40); Atka mackerel, the number of age 2 fish estimated by Lowe (1992) to be recruited into the fishery from 1972 to 1988; sablefish, the index of relative year class strength developed by McFarlane and Beamish (1992); Pacific hake, the number of age 2 fish estimated by Dorn and Methot (1989) that would be recruited into the fishery from each year class (Fig. 43); chub mackerel, the estimated number of catches of age 1 fish from 1976 to 1984 from MacCall et al. (1985) (Fig. 44); northern anchovy, the estimated abundance of age 0 fish recruited to the fishery from 1966 to 1988 from Jacobson and Lo (1989) (Fig. 46).

The sum of the nondimensional indices (Fig. 47A) shows that from 1962 to 1975, there was a below-average number of strong year classes. The years 1977 and 1978 were years of exceptionally good year class production, with 1977 being the most exceptional in the 27-yr time series. Strong and weak year class production can also be examined by summing the number of species that had positive and negative anomalies for each year (Fig. 47B). From 1960 to 1986 there were at least four species in the data series for each year, from 1964 to 1984 there were at least six species, and from 1971 to 1982 there were between eight and 12 species. In 1977 there were 10 species with positive anomalies (above average year class production) and two species with negative anomalies. In 1978 there were nine with positive anomalies and three with negative anomalies. It is clear from both methods that 1977 and 1978 were years of exceptional fish production in the time series from 1960 to 1986.

It is important to remember that year class strength is rarely known until fish are recruited into a fishery. This period of prerecruitment varies for the species in this report as described. During the prerecruitment period, there must have been adequate food for growth and survival of the strong year classes produced from 1976 to 1978. This indicates that the carrying capacity of the ocean must have changed for the species that produced strong year classes, to be able to support them through the period of prerecruitment.

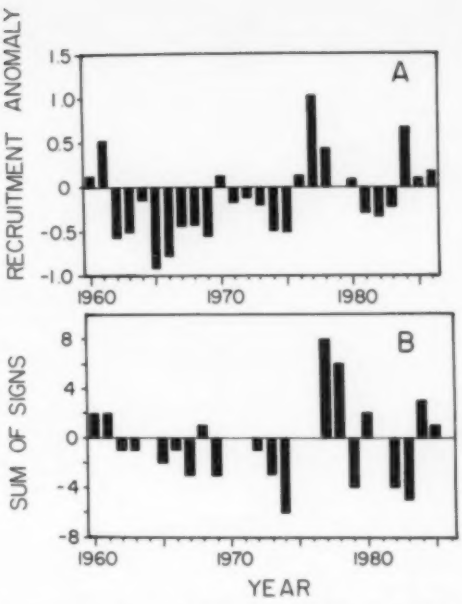

FIG. 47. (A) Anomalies of the sum of the nondimensional indices of recruitment strength of 12 nonsalmon species in this paper showing strong year class production in 1961, 1977, 1978, and 1984 and exceptional production in 1977 and (B) sum of the signs of the positive and negative anomalies showing that in 1977 and 1978, most of the species examined had stronger than average year class survival.

Discussion

In this report, I provided a relatively long list of examples of fisheries that were affected by the climate events in 1976 and 1977. Each example is quite short and omits detail and references about the biology and stock dynamics because the list of examples makes the paper rather long as it stands. The length of the list, however, indicates the magnitude of the impact the climate events in 1976 and 1977 had on fish production along the west coast of Canada and the United States. In another report (McFarlane and Beamish 1992), we examine the mechanisms responsible for strong year class production of one species, sablefish, and note the importance of the relationships among the intensity of the Aleutian Low pressure system, cooling in the central North Pacific Ocean, and calanoid copepod production.

The evidence in this paper strongly indicates that the period 1976–78, and 1977 in particular, was a time of exceptional productivity for fishes found off the Pacific coast of Canada and the United States. It appears that many species of fish in the Bering Sea and the more northern areas of the North Pacific Ocean had exceptionally strong year classes. However, there is evidence that some species that occur more to the south, particularly those off the coast of California, had improved survival also. The mechanisms responsible for the coast-wide effect on fish populations are poorly understood, but enough is known about the interrelations among climate and physical oceanography to speculate on the reasons for the production of the strong year classes that occurred for 1976–78.

The upper ocean heat storage (or the volume of surface layer water above the thermocline) is closely related to the magnitude of primary productivity (Chavez and Barber 1985; Lewis et al. 1988). The depth of the surface layer regulates productivity because ocean water below the thermocline is approximately 1000 times richer in nutrients than water above the thermocline (Redfield 1958). Climate-induced changes in heat storage affect the depth of the surface layer and are the principal mechanisms regulating primary and secondary productivity (Barber and Chavez 1983; Venrick et al. 1987). Heat loss from the surface waters and increased wind stress will increase vertical mixing, bringing nutrients into the euphotic zone. Reid (1962) proposed that horizontal divergence in the upper layer in the center of the Alaskan gyre brings nutrients into the mixed layer, and Thomson (1981) showed that the horizontal divergence transports this water towards the edge of the gyre. Thus, large productivity changes may result from changes in the depth of the euphotic zone. Beamish and Bouillon (1993) showed that there was a close association between an index of the Aleutian Low pressure system and the all-nation production of Pacific salmon. The smoothed trend of the index they developed changes shape in

1976, indicating a change towards more intense (low pressure) lows in the winter and spring. The intensification of the Aleutian Low beginning in 1976 would increase wind stress and upwelling offshore during the winter of 1976–77, resulting in increased nutrient supply to the surface that could be transported as nutrients, food, or both into rearing areas for young fishes.

The Southern Oscillation Index (SOI) is the difference between the normalized surface air pressure measured at Tahiti and Darwin (Trenberth 1984). El Niño – Southern Oscillation (ENSO) events are characterized by a relatively large positive anomaly followed by a relatively large negative anomaly. Extreme negative anomalies are described as El Niño events and are characterized by low pressures and wet, warm weather in the Northeast Pacific (van Loon and Madden 1981; Emery and Hamilton 1985) resulting in a warming of the surface temperature adjacent to the west coast of North America.

There is an atmospheric link between the intensity of an Aleutian Low and the SOI (Blackmon et al. 1983; Namias 1985). Modelling studies have demonstrated this link through a correlation between sea surface temperatures in the tropics and the North Pacific circulation. Variability in climate and ocean conditions in the Bering Sea is related to ENSO events, and the mechanism for the connection appears to be the Aleutian Low (Niebauer 1988). Nitta and Yamada (1989) and Trenberth (1990) showed that major atmospheric changes occurred in 1976 which persisted for at least a decade (Trenberth 1990). K.E. Trenberth and J.W. Hurrell (unpublished data) used an index of the Aleutian Low pressure system to demonstrate a strong relationship between atmosphere–ocean events in the North Pacific and the tropical Pacific where there was evidence of changes that were precursors for events in the North Pacific. The events in the tropics are associated with changes in higher latitudes through a process called teleconnections (Horel and Wallace 1981). Mysak (1986) noted that intensified Aleutian Lows frequently follow ENSO events but the intensity of the Aleutian Low is not necessarily related to the intensity of the ENSO event and not all ENSO events are followed by intense Aleutian Lows. The 1976–77 El Niño event (Bradley et al. 1987), therefore, is linked with the intense Aleutian Low through the atmosphere, and it is this atmosphere linkage that appears to be associated with midocean production changes.

Venrick et al. (1987) identified a significant increasing trend in total chlorophyll *a* in the central North Pacific Ocean. Chlorophyll *a* almost doubled from 1968 to 1985 and concentrations since 1980 were significantly higher than those before 1974. They detected increases after 1968 but were not able to determine if the increase was continuous or if a rapid change had occurred after 1980. According to their fig. 1, they had only one observation in 1978 and none in 1979, so it was not possible to determine if abrupt changes occurred before 1980. They believed the increases in productivity resulted from large-scale surface cooling combined with increased wind stress. They showed that the average sea level pressure in the winter months north of 25°N was lower from 1980 to 1985 than from 1968 to 1973. This extensive area of low pressure indicated to them that the increase in chlorophyll *a* occurred throughout the North Pacific.

As mentioned, copepod production at Ocean Station "P" (50°N, 145°W, Fulton 1983) was significantly related to the Aleutian Low Pressure Index (McFarlane and Beamish 1992; Beamish and Bouillon 1993). It is known that there was a major increase in zooplankton in the central subarctic Pacific in the 1980s (Brodeur and Ware 1992), suggesting that the abundance of copepods measured at Ocean Station "P" was indicative of a large-scale increase beginning in 1976. Calanoid copepods constitute most of the zooplankton biomass in the epipelagic zone of the subarctic Pacific (Miller et al. 1984). Two species, *Neocalanus plumchrus* and *N. cristatus*, make up most of the copepod biomass (Miller et al. 1984). They are in greatest abundance at the surface from April to July. After this period, they move into deep water where they spawn during winter. The reproductive cycle of this abundant plankton coincides with the reproductive cycle of many fishes, and it is not surprising, therefore, that larval copepods are a major source of food for larval and juvenile fishes.

How the strong production of copepods occurred is less clear. The strong copepod year class could result from an abundance of suitable food for larval copepods, or copepod fecundity might have improved as a result of environmental conditions. However, copepod abundance in one year did not correlate well with copepod abundance in the next year, suggesting that improved copepod production occurs because of improved larval copepod survival. Therefore, the climate and resulting ocean conditions probably served to improve both larval copepod survival and to concentrate copepods along the North American coast.

Climate changes in the North Pacific Ocean can affect the strength of the California Current from the north (Wickett 1967; Bernal 1980; Bernal and McGowan 1981) and from the south as a result of El Niño events (Namias 1985). Because fluctuations in the strength and nutrient content of the California Current affect zooplankton volume off the coast of California more than does coastal upwelling (Chelton et al. 1982), a strong California Current that occurs when the Aleutian Low is intense could produce increases in plankton and these increases are believed to persist for 1–3 yr (Chelton 1981). The El Niño event of 1976–77 could influence the productivity of fish stocks through changes in upwelling along the coast of California (Namais 1985) and by affecting the major winter climate system in the North Pacific. A major change in climate can, therefore, affect productivity of waters from California to the Bering Sea. Hollowed (1990) recently concluded that there was a general relationship between the occurrence of strong year classes and ocean conditions, suggesting that there was a link with large-scale ocean circulation patterns.

The response of fish populations from 1976 to 1978 clearly shows that the environment affects recruitment and cannot be ignored when studying the dynamics of fish populations. The large increases in abundance that occurred for some species from small spawning stocks indicated that stock-recruitment relationships for these and probably other species discussed in this paper can be closely related to changes in the environment. The changes in abundance were large and abrupt and had obvious impacts on the management of fisheries, yet it was a number of years before it was known that such a large and sudden change occurred. The abundance changes clearly demonstrate that physical changes in the ocean can affect the population dynamics of fishes with very different life histories, almost simultaneously, over a vast area. A coastwide, synchronous increase in year class strength of a number of species is a welcome event for fishermen and fisheries managers. However, it is to be expected that there will be periods when environmental conditions result in poor survival and managers need to recognize when these conditions are occurring and make timely adjustments to fishing plans that recognize that the changes in abundance are occurring for natural

reasons. It is also to be expected that similar events have occurred in the past and that the explanations for the abundance changes that appear in the literature may need to be revisited.

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